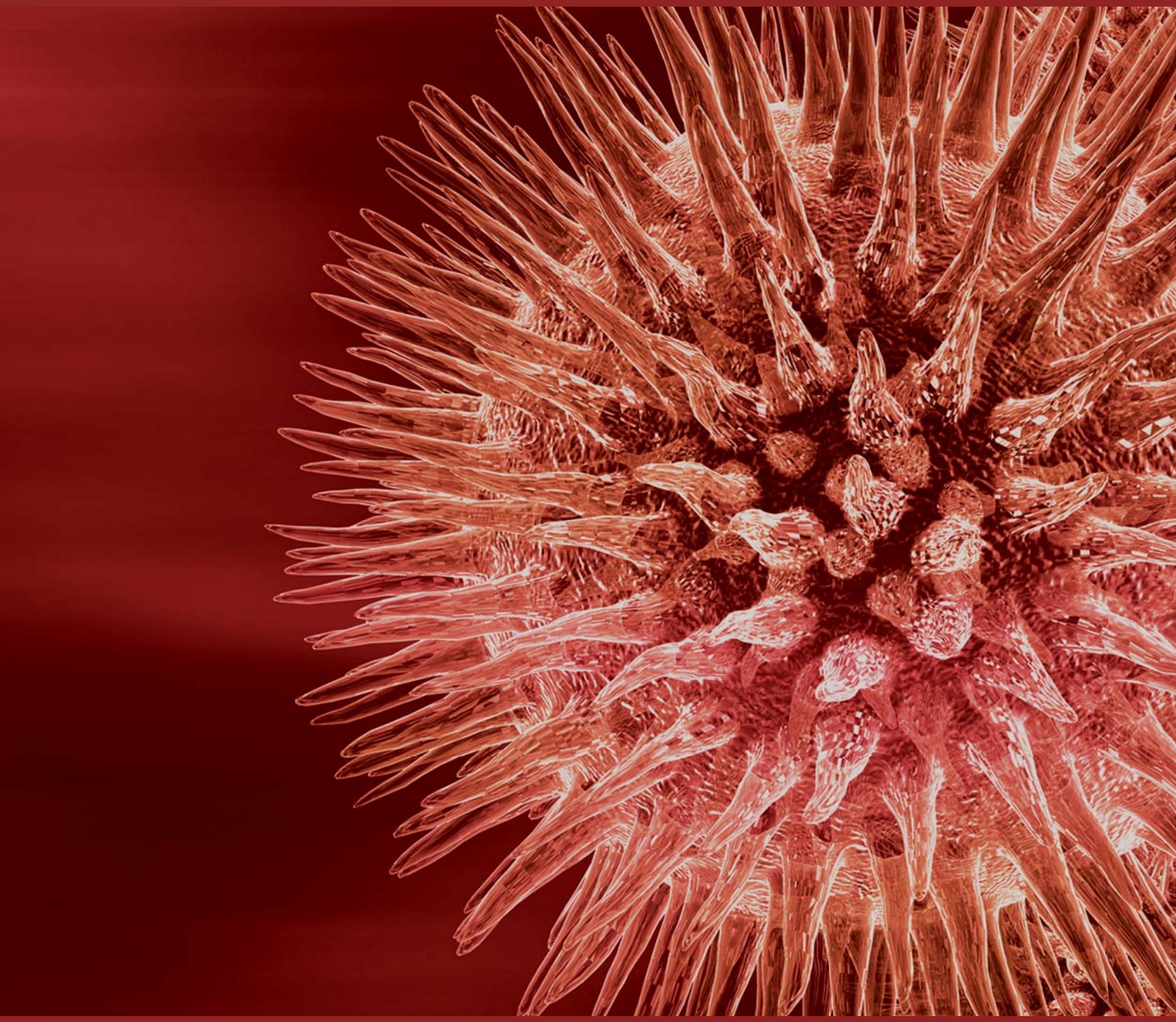
Journal of Biomedicine and Biotechnology

Natural Killer Cells in Healthy and Diseased Subjects

Guest Editors: Roberto Biassoni, John E. Coligan, and Lorenzo Moretta



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Editorial

Natural Killer Cells in Healthy and Diseased Subjects

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Natural killer cells or NK cells have been known since the 70s on the basis of their functional properties [1, 2], but essentially no molecular markers existed able to specifically identify them. Still in the early eighties, the few scientists that worked in NK cells field had difficulty convincing about the existence of those cells while, in the same period, immunologists were essentially in the search of the δ-chain of the T-cell receptor.

Nowadays, following the molecular characterization of different surface receptors and the signalling pathways as well as the characterization of the molecules specifically recognized and the analysis of their 3D-structures, the research on NK cells has become a highly competitive field. Researchers studying different functional and molecular aspects have been challenged to understand the function of these cells and the mechanism of the regulation of their function in terms of NK cell licensing or education and NK memory. In addition, it is always more complex the crosstalk with other cell of the immune system either by cell contact or through the release of soluble factors. Briefly, natural killer cells are responsible for the immune responses against tumor or virally infected cells. Their function is tightly regulated by a clonal and stochastic distribution of germline-encoded cell surface receptors, and these molecules are able to deliver either inhibitory or activating signals. Thus, every NK cell is equipped by at least a single inhibitory receptor which is functionally dominant and that senses the level of surface expression of MHC class I molecules on autologous cells mediating self-tolerance [3–15]. This function has been maintained during mammalian evolution by expansion of different multigene families coding for receptors with marked structural divergences that have been evolved in the different species together with appropriate MHC molecules serving as ligand [16]. Any alteration on target cell of the surface expression level of the MHC class I molecules, induced by viral infection or tumor transformation, induces NK-mediated cell killing. NK cell receptors evolved in a highly dynamic fashion, primarily driven by the necessity to deal with a large variety of pathogens and to recognize properties of cells characteristic of tumor transformation.

In this special issue, a series of reviews or articles on natural killer cells are published and summarize information related to NK cells and diseases and the rationale use of these knowledge for clinical applications. In detail we summarize the molecular and structural analysis of NK receptor and their interaction with ligands and how the diversity of KIR genotype and of HLA class I molecules are related with the outcome of a number of key human infections [17, 18]. Other contributions are based on the analysis of the knowledges regarding the molecular interactions between NK cells and myeloid antigen-presenting cells and their role in the regulation/polarization of adaptive immune responses [19, 20]. The ability of distinct species of gut-derived commensal bacterial to differently affect the outcome of DC/NK crosstalk and the Th1 polarization of the adaptive immune response is also discussed [19, 20]. The complex network of interactions involving the immune system is also through the use of cytokines. The functional redundancy and the specific role of the common gamma-chain cytokine family (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) in the regulation of the immune response and in the homeostasis of the lymphoid cells and the acquisition of memory-like functions have been analyzed [21]. In addition, novel strategies to deliver cytokines and the use of immunokine aimed to maximize their therapeutic

potential and to costimulate the NK cell activation by enhancing NK cell adhesion to target cells have been described [21, 22]. Several reviews analyze the mechanism of recognition used by NK cells to sense viral pathogens, like HCV, HBV, HIV, CMV, and other infectious diseases [1, 23–25] and the potential role of NK cells during severe sepsis and septic shock [26]. Cytomegaloviruses evolved different mechanisms to hide and survive to immune-related responses. Innate immunity is the first line of defence in the case of CMV infections, and viral particles are known to modulate the expression of ligand molecules specific for NK-triggering receptor on the surface of infected cells. In addition, some viral genes are also known to encode MHC class I-like surface molecules contributing to immunoevasion both in mice and in humans [23, 24]. Since CMVs and their respective hosts coevolved, it is tempting to speculate that the viral MHC class-I-like molecules originate from the host genomes. Revilleza et al. on the bases of nucleotides, proteins, and crystallographic/modelling structural data obtained from human and mice speculate that a single ancient event of partial genome transfer and not multiple phenomena may have evolved the different CMV class I-like molecules [23]. In various mice strains the capture of host-genome has originally allowed the development of MHC class-I-like molecules able to interact with inhibitory Ly49 receptors. Instead B6 and Ma/My have evolved activating Ly49 molecules, which through the interaction with viral class I-like molecules and/or with the presence of additional factors are able to trigger NK cell cytolysis rather than inhibit and probably to contribute to the development of NK cell memory [24]. Thus it appears clear that there is a strict interplay among NK cells, their receptors and the responses toward viral pathogens. To address these issues, we have an depth analysis discussing the different triggering receptors expressed by NK cells, their level of expression and the possible ligands involved in the interaction with cells infected by viruses belonging to different families (Orthomyxo-, Paramyxo-, Flavi-, Lentiviridae) [25]. Thanks to the latest review in which we have found discussed the NK-mediated responses following viral pathogen infection, here we find some recent points of view about how also microbial sepsis and the "systemic inflammatory response syndrome" or peptic shock may be related with NK cell-immune responses [26]. All the information regarding NK receptors and their ligands, as well as the fine regulation of innate/acquired immune-responses orchestrated by NK cells and the cytokines network are the basis for better use in cancer therapy. The NK cell responses toward tumor using specific mAbs and an ADCC mechanism and all attempts to increase anti-tumor cell cytotoxicity using cytokine combinations, TLR agonist and immunokines are also discussed [27, 28]. ADCC is often impaired in PBMC from patients with advanced cancer as a result of NK cell dysfunction probably generated by tumor-produced soluble factor(s) (i.e., TGFβ), thus any approaches aimed to increase the ADCC killing are necessary to obtain an efficient tumor cell readication.

Along this line, IL15, IL2 and the use of Lenalidomide, an analog of thalidomide known to increase FcγR-mediated signalling, adoptive cell transfer and ex-vivo cell expansion, have been successfully used [28]. Although, the firsts attempts in the use of autologous ex vivo expanded cells did not give encouraging results, it is now clear the basis of these failures, making allogeneic cell transfer more attractive. The use of KIR/HLA class I-mismatched cells, selected in order to be not sensitive to inhibitory signals from the recipient HLA ligands, have been proved to be protective against disease relapses [28].

The other face of the coin of NK cells and tumor is shown by Schmitt et al, that focalizes our attention on a rare form of hematolymphoid tumors including the extranodal NK/T-cell lymphoma (NKTCL) that is considered inside the provisional group of chronic NK-cell lymphoproliferative disorder. This lymphoma is apparently associated with EBV-associated malignancy with very poor prognosis, rare in western countries, but it represents at least 10% of non-Hodgkin's lymphomas in Asia and Central/South America [29]. Finally, two reviews are taking care of using the combined analysis of genetic data regarding KIR and HLA class I molecules and their association in autoimmune diseases and the cytokines network below the pathogenesis of chronic inflammation [30, 31]. In both cases, a decrease of the strong inhibitory pathways controlled by HLA-C-specific receptors and thus a reduced activating threshold promote immune responses, and this effect of changing the threshold of immune-suppression/activation may contribute to explain the genetic susceptibility of different autoimmune diseases.

In the last years, we have assisted to an explosion in the research field centered on NK cells. In the near future, we will see the publication of different results in the regulation of receptors and ligands expression by miRNA, as exemplified by the recent published article regarding the control of HLA-C expression in HIV infection [32]. There are different arguments that need a detailed study. Among these, we also know that KIR recognizes HLA class I ligands on the basis of the presence of particular amino acid residues; probably different exceptions exist. In addition, very little information is available about the role of viral peptides, presented by HLA class I able to trigger the activation of Killer Ig-like Receptors (KIR2DS, KIR3DS). The absence of such information might explain the failure, so far, of an experimental identification of ligands specific for different triggering KIR, with a single exception [33]. Finally, a complete characterization of the molecules recognized by all the cloned NK cells receptors is still needed to analyze in detail all the triggering pathways induced by NK cell-mediated recognition.

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References

[1] R. B. Herberman, M. E. Nunn, H. T. Holden, and D. H. Lavrin, "Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells," *International Journal of Cancer*, vol. 16, no. 2, pp. 230–239, 1975.

[2] R. Kiessling, E. Klein, and H. Wigzell, ""Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype," *European Journal of Immunology*, vol. 5, no. 2, pp. 112–117, 1975.

[3] E. Ciccone, O. Viale, D. Pende et al., "Specific lysis of allogeneic cells after activation of CD3- lymphocytes in mixed lymphocyte culture," *Journal of Experimental Medicine*, vol. 168, no. 6, pp. 2403–2408, 1988.

[4] H. G. Ljunggren and K. Karre, "In search of the 'missing self': MHC molecules and NK cell recognition," *Immunology Today*, vol. 11, no. 7, pp. 237–244, 1990.

[5] L. Moretta, E. Ciccone, A. Moretta, P. Hoglund, C. Ohlén, and K. Karre, "Allorecognition by NK cells: nonself or no self?" *Immunology Today*, vol. 13, pp. 300–306, 1992.

[6] W. M. Yokoyama and W. E. Seaman, "The Ly-49 and NKR-P1 gene families encoding lectin-like receptors on natural killer cells: the NK gene complex," *Annual Review of Immunology*, vol. 11, pp. 613–635, 1993.

[7] N. Wagtmann, R. Biassoni, C. Cantoni et al., "Molecular clones of the p58 NK cell receptor reveal immunoglobulin-related molecules with diversity in both the extra- and intracellular domains," *Immunity*, vol. 2, no. 5, pp. 439–449, 1995.

[8] A. D'Andrea, C. Chang, K. Franz-Bacon, T. McClanahan, J. H. Phillips, and L. L. Lanier, "Molecular cloning of NKB1 a natural killer cell receptor for HLA-B allotypes," *Journal of Immunology*, vol. 155, no. 5, pp. 2306–2310, 1995.

[9] M. Colonna and J. Samaridis, "Cloning of immunoglobulin-superfamily members associated with HLA-C and HLA-B recognition by human natural killer cells," *Science*, vol. 268, no. 5209, pp. 405–408, 1995.

[10] A. Moretta, C. Bottino, M. Vitale et al., "Receptors for HLA class-I molecules in human natural killer cells," *Annual Review of Immunology*, vol. 14, pp. 619–648, 1996.

[11] E. O. Long and N. Wagtmann, "Natural killer cell receptors," *Current Opinion in Immunology*, vol. 9, no. 3, pp. 344–350, 1997.

[12] P. E. Posch, F. Borrego, A. G. Brooks, and J. E. Coligan, "HLA-E is the ligand for the natural killer cell CD94/NKG2 receptors," *Journal of Biomedical Science*, vol. 5, no. 5, pp. 321–331, 1998.

[13] R. Biassoni, "Human natural killer receptors, co-receptors, and their ligands," *Current Protocols in Immunology*, no. 84, pp. 14.10.1–14.10.40, 2009.

[14] M. Carretero, C. Cantoni, T. Bellon et al., "The CD94 and NKG2-A C-typelectinscovalently assemble to form a natural killer cellinhibitoryreceptor for HLA class I molecules," *European Journal of Immunology*, vol. 27, no. 2, pp. 563–567, 1997.

[15] F. Borrego, M. Ulbrecht, E. H. Weiss, J. E. Coligan, and A. G. Brooks, "Recognition of human histocompatibility leukocyte antigen (HLA)-E complexed with HLA class I signal sequence-derived peptides by CD94/NKG2 confers protection from natural killer cell-mediated lysis," *Journal of Experimental Medicine*, vol. 187, no. 5, pp. 813–818, 1998.

[16] R. Biassoni, E. Ugolotti, and A. de Maria, "Comparative analysis of NK-cell receptor expression and function across primate species: perspective on antiviral defenses," *Self Non Self* , vol. 1, no. 2, pp. 103–113, 2010.

[17] K. M. Jamil and S. I. Khakoo, "KIR/HLA interactions and pathogen immunity," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 298348, 9 pages, 2011.

[18] M. G. Joyce and P. D. Sun, "The structural basis of ligand recognition by natural killer cell receptors," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 203628, 15 pages, 2011.

[19] O. Chijioke and C. Münz, "Interactions of human myeloid cells with natural killer cell subsets in vitro and in vivo," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 251679, 7 pages, 2011.

[20] V. Rizzello, I. Bonaccorsi, M. L. Dongarr, L. N. Fink, and G. Ferlazzo, "Role of natural killer and dendritic cell crosstalk in immunomodulation by commensal bacteria probiotics," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 473097, 10 pages, 2011.

[21] R. Meazza, B. Azzarone, A. M. Orengo, and S. Ferrini, "Role of common-gamma chain cytokines in NK cell development and function: perspectives for immunotherapy," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 861920, 16 pages, 2011.

[22] L. Kühne, M. Konstandin, Y. Samstag, S. Meuer, T. Giese, and C. Watzl, "WF10 stimulates NK cell cytotoxicity by increasing LFA-1-mediated adhesion to tumor cells," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 436587, 6 pages, 2011.

[23] M. R. Revilleza, R. Wang, J. Mans, M. Hong, K. Natarajan, and D. H. Margulies, "How the virus outsmarts the host: function and structure of cytomegalovirus MHC-I–like molecules in the evasion Of natural killer cell surveillance," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 724607, 12 pages, 2011.

[24] M. Pyzik, E.-M. Gendron-Pontbriand, and S. M. Vidal, "The impact of Ly49-NK cell-dependent recognition of MCMV infection on innate and adaptive immune responses," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 641702, 9 pages, 2011.

[25] F. Marras, F. Bozzano, and A. De Maria, "Involvement of activating NK cell receptors and their modulation in pathogen immunity," *Journal of Biomedicine and Biotechnology*, vol. 2011, 2011.

[26] L. Chiche, J. -M. Forel, G. Thomas et al., "The role of natural killer cells in sepsis," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 986491, 8 pages, 2011.

[27] K. L. Alderson and P. M. Sondel, "Clinical cancer therapy by NK cells via antibody-dependent cell mediated cytotoxicity," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 379123, 7 pages, 2011.

[28] E. M. Levy, M. P. Roberti, and J. Mordoh, "Natural killer cells in human cancer: from biological functions to clinical applications," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 676198, 11 pages, 2011.

[29] C. Schmitt, N. Sako, M. Bagot, Y. Huang, P. Gaulard, and A. Bensussan, "Extranodal NK/T-cell lymphoma: toward the identification of clinical molecular targets," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 790871, 11 pages, 2011.

[30] P. K. Yadav, C. Chen, and Z. Liu, "Potential role of NK cells in the pathogenesis of inflammatory bowel disease," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 348530, 6 pages, 2011.

[31] S. Dunphy and C. M. Gardiner, "NK cells and psoriasis," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 248317, 10 pages, 2011.

[32] S. Kulkarni, R. Savan, Y. Qi et al., "Differential microRNA regulation of HLA-C expression and its association with HIV control," *Nature*, vol. 472, no. 7344, pp. 495–498, 2011.

[33] R. Biassoni, A. Pessino, A. Malaspina et al., "Role of amino acid position 70 in the binding affinity of p50.1 and p58.1 receptors for HLA-Cw4 molecules," *European Journal of Immunology*, vol. 27, no. 12, pp. 3095–3099, 1997.

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Review Article

KIR/HLA Interactions and Pathogen Immunity

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The innate immune system is the first line of defence in response to pathogen infection. Natural killer (NK) cells perform a vital role in this response with the ability to directly kill infected cells, produce cytokines, and cross-talk with the adaptive immune system. These effector functions are dependent on activation of NK cells which is determined by surface receptor interactions with ligands on target cells. Of these receptors, the polymorphic killer immunoglobulin-like receptors (KIRs), which interact with MHC class 1 (also highly polymorphic), are largely inhibitory, and exhibit substantial genetic diversity. The result is a significant variation of NK cell repertoire between individuals and also between populations, with a multitude of possible KIR:HLA combinations. As each KIR:ligand interaction may have differential effects on NK cell activation and inhibition, this diversity has important potential influences on the host response to infections. Genetic studies have demonstrated associations between specific KIR:ligand combinations and the outcome of viral (and other) infections, in particular hepatitis C and HIV infection. Detailed functional studies are not required to define the mechanisms underpinning these disease associations.

1. Introduction

Natural Killer (NK) cells are key effector cells of the innate immune system, and as such are crucial in the antiviral immune response. They are multifunctional, with an ability to interact directly with infectious agents, through pattern recognition receptors, with infected cells, via expressed cell surface receptors, and with cells of the adaptive immune system via cell-cell interactions and through secretion of cytokines [1]. Such cytokines are predominantly pro-inflammatory such as interferon (IFN)-γ or tissue growth factor (TGF)-β, but may in some cases be immunoregulatory [2].

NK cell activation is controlled by a complex balance between activating and inhibitory receptors such that the net signal derived from these receptors is integrated to determine whether or not NK cell effector functions are initiated [3–5]. Many of these receptors are monomorphic and expressed on all cells. Furthermore, they are relatively well conserved between human and mice. This particularly applies to the activating receptors, including NKp46 and NKG2D, which have been shown to be especially important in the host-pathogen interaction. The inhibitory receptors, however, fall into two main, although not exclusive, groups: the killer-cell immunoglobulin-like receptors (KIRs) and the NKG2 families. Both have major histocompatibility complex (MHC) class I ligands, but of different types. The NKG2 family, of which NKG2A is the main inhibitory member, is relatively nonpolymorphic and conserved throughout evolution. The functional receptor, a heterodimer of CD94 and NKG2A, binds human leucocyte antigen (HLA)-E plus signal peptides derived from classical HLA class I alleles [6]. Conversely, the KIRs are diverse and polymorphic, with polymorphic HLA class I ligands. Thus, whilst both families may be important in the immune response against pathogens, the KIR family are more likely to be responsible for generating diversity in the immune response to specific pathogens within the human population. They have received much attention as potential disease association markers for a number of infections which have discrete clinical outcomes. The aim of this paper is to summarize our current knowledge of how KIRs and KIR ligand diversity may influence the outcome of a number of key human infections.

2. Structure and Genetics

KIRs exhibit substantial diversity at both the allelic and haplotypic levels. Furthermore, their expression on NK cells is stochastic and variegated [7, 8]. The result is a diverse repertoire of NK cell clones within an individual and also substantial NK cell diversity between populations. Additionally, their HLA class I ligands are extremely polymorphic and this generates a further level of functional diversity. These factors likely synergize to generate varying susceptibility to pathogens and disease.

KIRs are encoded in a 150 kb region on chromosome 19q13.4 within the leukocyte receptor complex (LRC) [9]. There are at least 17 KIR genes or pseudogenes with substantial allelic diversity of many of these genes [10, 11]. Comparison of humans and nonhuman primates has revealed the rapid evolution of this locus, most likely through a combination of gene duplication and nonhomologous recombination [12]. This evolution is thought to be driven by selective pressure from exposure to pathogens, and also by the pressure for reproductive success [13]. The sum total of these effects is substantial genetic diversity at the level of the locus, which has been simplified into two main KIR haplotypes: "A" and "B" based on gene content. The B group of haplotypes are defined by the presence of one or more of the following genes: KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5, and KIR3DS1. Conversely, the group A haplotypes are defined by the absence of all of these genes and the presence of KIR2DS4 [14]. The two haplotypes are thought to have been maintained within the human population by balancing selection; however, the frequency of these haplotypes varies substantially between populations [15, 16]. In general, A haplotypes are associated with an improved response to pathogens, whereas B haplotypes are associated with improved reproductive fitness [17–19].

The KIR proteins are members of the immunoglobulin (Ig) superfamily of receptors. Their nomenclature is based on their structure, where the number of Ig-like extracellular domains (2D or 3D) and the length of the cytoplasmic tail (long, L or short, S) defines the name of the protein. The long cytoplasmic tails contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and confer an inhibitory signal when the receptors are engaged with their ligands. The short tailed receptors lack ITIMs and are activating. These activating receptors have a transmembrane lysine residue which allows binding to the immunoreceptor tyrosine-based activating motif (ITAM) containing adaptor DAP12. Thus, KIR2DL1 has two extracellular Ig-like domains, a long intracytoplasmic tail and transduces an inhibitory signal, whereas KIR3DS1 has three extracellular Ig-like domains and a short intracytoplasmic tail and associates with DAP12 to transduce an activating signal. An exception to this is KIR2DL4 which has a long intracytoplasmic tail, but associates with FcεR1γ to transduce an activating signal [20]. There is also diversity in the configuration of the extracellular domains, as for the two Ig domain KIRs the extracellular domains may be in a D1D2 (the majority) or D0D2 (KIR2DL4 and KIR2DL5) configuration. This extracellular domain organisation reflects evolutionary relationships, and thus the D0D2 2Ig domain KIRs comprise the Lineage I KIRs, the 3 IG domain KIRs excluding KIR3DL3 form the Lineage II KIRs and the D1D2 2Ig domain KIRs form Lineage III [21]. Lineage I is the most conserved of the lineages having representative members in the old world monkeys, whereas the Lineage III KIRs are not well conserved even between humans and the common chimpanzees.

3. KIR Ligands

That inhibitory KIRs interact with polymorphic HLA class 1 ligands is well established, and in general the Lineage III KIRs bind MHC-C. Thus, the inhibitory receptors KIR2DL1, 2DL2, and 2DL3 are specific for HLA-C: KIR2DL1 binds alleles of HLA-C with lysine at position 80 (HLA-C2), whereas KIR2DL2 and 2DL3 bind HLA-C alleles with asparagine at position 80 (HLA-C1) [22, 23]. The affinity of these interactions may differ [24], and it is thought that KIR2DL3:HLA-C1 is a relatively weak interaction, whilst KIR2DL2:HLA-C1 and KIR2DL1:HLA-C2 are relatively stronger [25]. Of the Lineage II KIRs, KIR3DL1 recognises HLA-B alleles with the Bw4 serological motif (HLA-Bw4) and also some HLA-A alleles also with the Bw4 motif; KIR3DL2 binds HLA-A3 and -A11 [26, 27]. The specific recognition of HLA class 1 by the activating KIRs is less well defined (Table 1), although sequence homology predicts they should have similar binding specificities as their inhibitory counterparts. Thus, binding of KIR2DS1 to HLA-C is of a similar specificity to that of KIR2DL1, but at a substantially lower affinity [28]. Furthermore, although KIR2DS2 and KIR3DS1 share substantial sequence homology with their inhibitory counterparts, KIR2DL2/3 and KIR3DL1, respectively, binding to the relevant ligands has not been convincingly established. One mitigating factor is that all the inhibitory KIRs tested to date have been shown to have selectivity for the peptide bound by MHC class I, and this implies that specific peptides, either host or viral, may modulate binding of KIRs to their MHC class I ligands. Thus, the absence of a defined HLA specificity may be because key (host or viral) peptides that determine the interaction have yet to be tested, or that they genuinely do lack a ligand [29].

As the gene cluster for HLA class I is located on chromosome 6, inheritance is unlinked to KIRs on chromosome 19. This generates diversity in the number of potential inhibitory interactions that the NK cells from a single individual may have. For instance, an individual with both group 1 and group 2 HLA-C alleles and also an HLA-Bw4 allele has the potential for three inhibitory KIR:HLA interactions, whereas an individual homozygous at the HLA-C locus and with no HLA-Bw4 alleles has only one inhibitory KIR:HLA interaction. Thus, in genetic studies it may be necessary to incorporate the KIR ligand interactions into a biological model, rather than just considering KIRs in isolation.

Within an individual, the KIRs, in combination with the CD94:NKG2A heterodimer, are expressed in a variegated pattern on NK cells. This generates an NK cell repertoire based on inhibitory receptors for self-MHC class I. The patterns of expression are complex, but have recently been segregated into five main types [8]. The relevance of an NK cell

Table 1: KIRs molecules and their HLA ligands.

KIRs	Ligand
2DL1	HLA-C2
2DS1	HLA-C2
2DL2	HLA-C1
2DL3	HLA-C1
2DS2	HLA-C1
2DL4	HLA-G
2DL5	Unknown
2DS3	Unknown
2DS4	HLA-A11, HLA-C
2DS5	Unknown
3DL1	HLA-Bw4
3DS1	HLA-Bw4^{80I}
3DL2	HLA-A3,11
3DL3	Unknown

repertoire may be similar to that of a T-cell repertoire. Thus, if a specific receptor:ligand combination is important for recognizing a host cell infected by a specific virus, then individuals who have a repertoire with more NK cells expressing that specific receptor may have a better immune response to that specific infection. Furthermore, NK cells that do not express a receptor for self-MHC class I appear to be relatively hypofunctional, which implies that they are likely to be less responsive to pathogens [30]. This is thought to be the result of selection and "licensing" processes that are dependent on these receptors during NK cell maturation [31, 32].

Thus, diversity within the KIR system is present at the level of the locus, of the allele, of the ligand, and within their expression patterns. It may be that all these factors can influence the host immune response to infection. We will now focus on how this diversity may impact the outcome some key human infections.

4. Hepatitis C Virus (HCV)

Infection with HCV leads to chronicity in the majority of cases. These individuals remain anti-HCV antibody positive with detectable HCV RNA, as compared to those who clear infection who remain antibody positive long after exposure, but have no detectable HCV RNA. Those with chronic infection have long-term sequelae that include cirrhosis and hepatocellular cancer (HCC). NK cells provide an early line of defence in the host response to HCV [33–35]. Furthermore, functional studies have demonstrated NK cell abnormalities in chronic infection, including decreased levels in the peripheral blood [36] and reduced cytotoxicity [37, 38]. This function can normalise with interferon-based treatment [39], and in patients where cytotoxicity is preserved, progression of liver fibrosis is reduced [36].

The categorization of HCV exposed individuals into those with and without detectable HCV RNA provides a simple variable which can be used to elicit genetic factors associated with protection against HCV. In a study of 1037 exposed individuals the compound KIR:HLA genotype KIR2DL3 and its ligand the group 1 HLA-C (HLA-C1) alleles was associated with resolution of infection. This was in a recessive model in which protection was found in those homozygous for both KIR2DL3 and HLA-C1, and then only in individuals exposed with relatively low inocula such as via intravenous drug use (IVDU) as compared to those exposed through infected blood products [40]. This finding was confirmed in a subsequent study of 160 individuals with IVDU as the risk factor for infection [41]. The finding that inhibitory interactions are protective against a viral infection initially seems counterintuitive. However, natural killer cells are held in check by their inhibitory receptors and, as originally proposed by the missing-self hypothesis, loss of these interactions is a key mechanism to permit NK cell activation [4]. KIR2DL3:HLA-C1 is a lower avidity interaction than KIR2DL2:HLA-C1 and also KIR2DL1:HLA-C2, and therefore the observed correlation with outcome of HCV infection is consistent with the binding data in that lower avidity interactions are more easily overcome than higher avidity ones [24, 25]. Although, HCV does not appear to substantially affect MHC class I expression, it has recently been found that NK cells are unexpectedly responsive to changes in the peptide repertoire of MHC class I through a mechanism of peptide antagonism. Therefore, it may be that this mechanism is more important in perturbing the KIR2DL3:HLA-C1 interaction than wholesale downregulation of MHC class I [42]. Functional data in influenza infection support this inhibitory receptor hierarchy. *In vitro* studies demonstrate that NK cells from individuals with the genotype KIR2DL3:HLA-C1 were activated more rapidly by autologous influenza infected targets than those from individuals with a KIR2DL1:HLA-C2 genotype [43]. The protective effects of KIR2DL3:HLA-C1 homozygosity have also been demonstrated for individuals who are exposed to HCV infection through high-risk behaviour, and do not have antibodies to HCV, or HCV RNA [44]. Furthermore, as KIRs are expressed on NK cells in a variegated manner, homozygosity may be protective because individuals with two copies of this gene have more NK cells expressing the protective KIRs. Indeed individuals that resolve HCV infection do have a higher frequency of NK cells expressing HLA-C-specific KIRs [33]. Additionally, individuals successfully treated with interferon-α-based regimens also have a higher frequency of KIR2DL3:HLA-C1, than those who do not make successful treatment responses [44, 45]. Thus, this gene combination has a consistently protective effect across several different scenarios within HCV exposure and infection. Recently, this protection has been mapped to the allelic level and it has been shown that HLA-Cw*07 (one of the KIR2DL3 ligands) was not protective against chronic infection, nor associated with a successful treatment response. This may be related to the education of NK cells from these individuals, as this allele has been shown to be associated with a "strong educator" phenotype, that is, NK cells from these individuals produce relatively large amounts of IFNγ compared to individuals with other HLA types [8]. Finally, HLA-C1 and also KIR3DS1 were found to be independently protective against the development of hepatocellular carcinoma in individuals with chronic HCV infection [46].

However, this simple model of genetic protection has not been found in all patient populations. KIR2DL3 is found on the "A" group of haplotypes, as is KIR2DS4. Consistent with this, KIR2DS4 has been associated with protection against chronic HCV infection [47]. Similarly, the B group of haplotypes are marked with KIR2DL5, and have been found to be associated with a poor response to treatment for HCV [48]. Finally, KIR2DL3:HLA-C1 was not found to be protective in a cohort of HIV/HCV coinfected individuals, implying that the presence of HIV infection modulates the protective effect of KIRs [49].

Due to the difficulty of obtaining samples in the acute phase of HCV infection, individuals have been studied predominantly in the chronic phase. Thus, it has been difficult to correlate these genetic effects with function. Furthermore, in the acute phase of infection, there are generally lower numbers of $CD56^{dim}$ NK cells which are the KIR expressing subpopulation, suggesting sequestration of these cells to the liver [33, 34]. Indeed, lower frequencies of peripheral blood NK cells expressing the key activating receptors NKp30, NKp46, and NKG2D are found in the acute phase of HCV infection in those that resolve infection compared to those that become chronically infected [33]. During chronic infection with HCV multiple changes in NK cell receptor expression have been observed, but in general KIR expression is low or normal, and NKG2A expression is increased [50–52]. One correlate is that CD107a expression on peripheral NK cells is increased on KIR2DL2/3+ NK cells in acute HCV infection compared to KIR2DL1+ NK cells and also compared to KIR2DL2/3+ NK cells from healthy donors [34].

5. Hepatitis B Virus (HBV)

Like HCV, HBV is a hepatotrophic virus that causes a major global health problem. An estimated 2 billion individuals have been infected with HBV and approximately 350 million are suffering from chronic disease [53]. Of those with chronic infection 25% die of the complications of HCC or cirrhosis, resulting in 600,000 deaths per year. NK cells are activated in the early response to infection, and there is substantial population variability in the rates of HBV infection [54]. Whilst detailed genetic and functional analyses exploring KIR-HLA influences on HBV in large cohorts of HBV are lacking, Lu et al. analysed the KIR genes in 150 patients with chronic HBV infection (CHB), 251 spontaneous resolvers, and 451 healthy controls. They found a lower frequency of the A haplotype, and higher frequency of the B haplotype in patients exposed to hepatitis B compared with healthy controls, implying a susceptibility effect of the B haplotype [18]. A second study, comparing 182 CHB patients with 140 healthy controls, was consistent with this observation [55]. The authors reported that KIR2DL3:HLA-C1 homozygosity was protective, and KIR2DL1:HLA-C2 was associated with susceptibility to HBV infection. Thus, there are important similarities between hepatitis C and hepatitis B infections, despite these viruses being phylogenetically unrelated.

6. HIV

HIV was the first viral infection for which an association of KIRs with outcome was observed. In a seminal study performed by Martin et al. [56], it was shown that the activating receptor KIR3DS1 was associated with a beneficial effect in HIV in combination with HLA-B alleles that have the Bw4 serological epitope with isoleucine at position 80 ($HLA\text{-}Bw4^{80I}$). This epistatic interaction protected against a decline in CD4 count, and hence the development of AIDS. Subsequent work from the same group has also shown that this combination delays the onset of opportunistic infection and is associated with a slightly lower viral load [56–58]. Overall this association forms an attractive model as the activating receptor-ligand interaction could be associated with enhanced NK cell reactivity, and, hence, an improved antiviral immune response. Furthermore, HIV has been shown to selectively downregulate HLA-A and -B, but not HLA-C, implying that it may target this specific interaction [59]. However, to date it has been difficult to demonstrate clearly that KIR3DS1 binds $HLA\text{-}Bw4^{80I}$ alleles in binding assays. This is not unexpected as the avidity of activating KIRs for MHC class I are significantly lower than for the inhibitory KIRs with similar predicted binding specificity [60, 61]. Furthermore, the peptide bound by the MHC class I molecule can profoundly influence the interaction of KIRs with MHC class I [28, 42, 62]. This complexity in binding may be one explanation for differences between these and other studies, such as that of Gaudieri et al. in which KIR3DS1 was not noted to be protective but specific HLA-B alleles were [63]. Additionally, Boulet et al. found a higher prevalence of KIR3DS1 in a cohort of injection drug users that remained HIV seronegative as compared to a matched seropositive cohort [64]. However, they found no association with specific HLA-B alleles in this relatively small cohort. Notwithstanding this, Alter et al. have demonstrated significant inhibition of viral replication in HIV-infected $HLA\text{-}Bw4^{80I}$-positive T cells when cultured with KIR3DS1+ NK cells [65]. This finding was supported in a subsequent study demonstrating increased IFNγ production and CD107a upregulation in HIV-infected individuals with the KIR3DS1, but not specifically the KIR3DS1/$HLA\text{-}Bw4^{80I}$ compound genotype [66].

In addition to a model based on activating receptor-ligand interactions, a hierarchy of inhibitory receptor-ligand interactions may also be associated with outcome of HIV infection. For the most part KIR3DL1 and KIR3DS1 segregate as alleles at a single locus. KIR3DL1 alleles are also extremely polymorphic with more than 50 alleles described [67]. These alleles are associated with different levels of expression of the KIR3DL1 allele and have been correlated with outcome of HIV infection [68, 69]. Martin et al. found that the high-expressing KIR3DL1 alleles combined with HLA-B*57 (a $HLA\text{-}Bw4^{80I}$ allele) was the most protective combination against progression of HIV. In this analysis, it was more protective than KIR3DS1 in combination with $HLA\text{-}Bw4^{80I}$. However, the most protective KIR allele was KIR3DL1*004, which is not expressed at the cell surface. The authors rationalise this on the basis of a potential

intracellular interaction of KIR3DL1*004 either with its ligand, in a manner similar to the endosomal interaction of KIR2DL4 with HLA-G, or with other NK cell receptors [70]. The protective effects of high expressing KIR3DL1 alleles and HLA-B*57 were subsequently independently confirmed at an immunogenetic level, and functional data from this same group has shown that KIR3DL1+ NK cells from IVDUs with a HLA-Bw4 ligand have enhanced functionality, as determined by CD107a expression as well as IFNγ and TNFα secretion [64, 71].

Whilst HLA-B has been associated with the outcome of HIV infection in a number of studies, recent genome-wide association studies have also highlighted the relevance of HLA-C in determining the viral set point and also in defining "elite controllers" of HIV infection [72, 73]. Whilst HLA-C can present HIV-derived peptides to T cells [74], this association may be related to epistatic interactions with KIRs. To date large studies have not defined a protective combination of specific KIRs and HLA-C allotypes, although they have been reported to be protective against HIV-1 transmission in a relatively small study of African sex workers [75]. Nevertheless, the absence of genetic associations does not mean that the HLA-C specific KIRs have no role in controlling HIV infection. If both HLA-C1 and HLA-C2 conferred similar degrees of protection against HIV, their role would not be revealed by the broad consideration of KIRs and HLA-C1 and HLA-C2 interactions. In depth allelic analysis may therefore be required. The -35 C/T polymorphism at the HLA-C locus, identified as protective in the genome-wide association study of Fellay et al., is associated with the level of HLA-C expression, and higher levels of HLA-C expression have also been associated with slower progression to AIDS and improved viral control [72, 76]. This is reminiscent of the effects of KIR3DL1 expression, and it may be that there are differences in NK cell education between high and low HLA-C expressers, which are reflected in their antiviral activity. Alleles of HLA-Cw*07, which are associated with the "strong educator" phenotype, have the nonprotective -35 polymorphism (a "T" allele), are generally expressed at low levels and are associated with the most rapid progression of disease [76]. Conversely, alleles expressed at high levels tend to have the "C" allele and are associated with slower progression. Thus, HIV illustrates the complexity of how KIRs and MHC may interact to determine the outcome of HIV infection, with the potential to generate multiple models to explain the immunogenetic findings.

7. Other Infections

KIRs have been implicated most strongly in HCV and HIV infections, which to some extent reflects the focus of the immunogenetic community on these important diseases. These pathogens cause chronic infection with readily measurable outcomes which can be used to stratify individuals into disease phenotypes. The observation that KIR genotype is important for the response to influenza *in vitro* implies that KIRs may also be important for other viral infections and that they provide a selective advantage against pathogens that may cause acute disease only.

Additionally, NK cells are thought to be particularly relevant for viral infections of the herpes family [77]. Thus, although CMV causes a latent infection in the majority of infected individuals, in immunocompromised individuals it can reactivate, and cause a life-threatening illness. In particular, it can severely compromise individuals undergoing bone marrow transplantation (BMT). Despite substantial variability in protocols for this procedure, a consistent finding appears to be that having more donor activating KIR genes helps to prevent CMV reactivation [78–80].

Activating KIRs genes are also associated with protection against human papilloma virus (HPV) in the relatively unusual setting of recurrent respiratory papillomatosis (RRP), which is due to an impaired immune response to HPV. In one study, protection was associated with KIR2DS1, KIR2DS5, and KIR3DS1 [81]. Interestingly, KIR3DS1 is positively associated with the development of another HPV-associated disease, cervical neoplasia [82]. The authors suggest that the increased activation of NK cells leads to chronic inflammation and, hence, cancer. Thus, these two studies are consistent with each other in that it appears that an absence of KIR3DS1+ NK cells permits active replication of HPV, whereas in cervical neoplasia the activity of KIR3DS1+ NK cells, in the absence of viral eradication, leads to ongoing inflammation.

Like CMV, herpes simplex virus (HSV) causes an asymptomatic infection in the majority of individuals. However, symptomatic infection may occur in relatively immunocompetent individuals. The KIR genes, KIR2DL2 and KIR2DS2, which are in tight linkage disequilibrium, were associated with asymptomatic HSV infection [83]. However, due to this tight linkage, it was impossible to determine whether it was the activating or the inhibitory receptor that was associated with a poor response.

In addition to their role in viral infections, NK cells may also affect the response to protozoa [84], and there may also be a role for KIRs in affecting outcome. In a study of 23 individuals, those with the allele KIR3DL2*002 secreted higher levels of IFNγ in response to Plasmodium falciparum-infected red blood cells [85]. As red blood cells are MHC class I deficient, this may reflect an influence of KIR:HLA on NK cell-macrophage cross-talk, as opposed to any direct interaction between NK cells and infected red blood cells [85, 86]. Alternatively, it may be an effect of a gene in linkage with KIR3DL2*002 or related to differential education of NK cells from individuals with this haplotype. Thus, in these diverse infections KIR genetics have the potential to influence outcome. Larger and more definitive studies are required.

8. Summary

Natural killer cells are important players in an effective antiviral immune response. Their expression of multiple cell surface receptors implies that during different infections different receptors are likely to be important. Many of these receptors are monomorphic and expressed on all NK cells. KIRs have a variegated expression pattern, and their complex genetics coupled with their HLA class I ligands imply that they are involved in generating population diversity in the

antiviral immune response. Tenable models based on both activating and inhibitory receptor-ligand interactions have been generated by detailed genetic studies involving large cohorts. Whilst functional studies have shed some light on these associations, the molecular mechanisms underpinning these genetic models still requires fine tuning.

Abbreviations

NK: Natural killer
IFN: Interferon
TGF: Growth factor
KIRs: Killer immunoglobulin-like receptor
MHC: Major histocompatibility complex
HLA: Human leucocyte antigen
Ig: Immunoglobulin
ITIM: Immunoreceptor tyrosine-based inhibitory motif
ITAM: Immunoreceptor tyrosine-based activating motif
LRC: Leukocyte receptor complex
HCV: Hepatitis C virus
HCC: Hepatocellular cancer
HBV: Hepatitis B virus
HIV: Human immunodeficiency virus
CMV: Cytomegalovirus
HPV: Human papilloma virus
RRP: Respiratory papillomatosis
HSV: Herpes simplex virus.

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References

[1] A. Horowitz, R. H. Behrens, L. Okell, A. R. Fooks, and E. M. Riley, "NK cells as effectors of acquired immune responses: effector $CD4^+$ T cell-dependent activation of NK cells following vaccination," *Journal of Immunology*, vol. 185, no. 5, pp. 2808–2818, 2010.

[2] A. De Maria, M. Fogli, S. Mazza et al., "Increased natural cytotoxicity receptor expression and relevant IL-10 production in NK cells from chronically infected viremic HCV patients," *European Journal of Immunology*, vol. 37, no. 2, pp. 445–455, 2007.

[3] A. Moretta, G. Tambussi, C. Bottino et al., "A novel surface antigen expressed by a subset of human $CD3^-CD16^+$ natural killer cells. Role in cell activation and regulation of cytolytic function," *Journal of Experimental Medicine*, vol. 171, no. 3, pp. 695–714, 1990.

[4] H. G. Ljunggren and K. Karre, "In search of the 'missing self': MHC molecules and NK cell recognition," *Immunology Today*, vol. 11, no. 7, pp. 237–244, 1990.

[5] P. Parham, "MHC class I molecules and KIRS in human history, health and survival," *Nature Reviews Immunology*, vol. 5, no. 3, pp. 201–214, 2005.

[6] V. M. Braud, D. S. J. Allan, C. A. O'Callaghan et al., "HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C," *Nature*, vol. 391, no. 6669, pp. 795–799, 1998.

[7] H. G. Shilling, L. A. Guethlein, N. W. Cheng et al., "Allelic polymorphism synergizes with variable gene content to individualize human KIR genotype," *Journal of Immunology*, vol. 168, no. 5, pp. 2307–2315, 2002.

[8] M. Yawata, N. Yawata, M. Draghi, F. Partheniou, A. M. Little, and P. Parham, "MHC class I specific inhibitory receptors and their ligands structure diverse human NK-cell repertoires toward a balance of missing self-response," *Blood*, vol. 112, no. 6, pp. 2369–2380, 2008.

[9] H. Wende, M. Colonna, A. Ziegler, and A. Volz, "Organization of the leukocyte receptor cluster (LRC) on human Chromosome 19q13.4," *Mammalian Genome*, vol. 10, no. 2, pp. 154–160, 1999.

[10] R. J. Boyton and D. M. Altmann, "Natural killer cells, killer immunoglobulin-like receptors and human leucocyte antigen class I in disease," *Clinical and Experimental Immunology*, vol. 149, no. 1, pp. 1–8, 2007.

[11] S. I. Khakoo and M. Carrington, "KIR and disease: a model system or system of models?" *Immunological Reviews*, vol. 214, no. 1, pp. 186–201, 2006.

[12] F. Canavez, N. T. Young, L. A. Guethlein et al., "Comparison of chimpanzee and human leukocyte Ig-like receptor genes reveals framework and rapidly evolving genes," *Journal of Immunology*, vol. 167, no. 10, pp. 5786–5794, 2001.

[13] P. Parham, L. Abi-Rached, L. Matevosyan et al., "Primate-specific regulation of natural killer cells," *Journal of Medical Primatology*, vol. 39, no. 4, pp. 194–212, 2010.

[14] S. G. E. Marsh, P. Parham, B. Dupont et al., "Killer-cell immunoglobulin-like receptor (KIR) nomenclature report, 2002," *Human Immunology*, vol. 64, no. 6, pp. 648–654, 2003.

[15] S. E. Hiby, J. J. Walker, K. M. O'Shaughnessy et al., "Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success," *Journal of Experimental Medicine*, vol. 200, no. 8, pp. 957–965, 2004.

[16] S. E. Hiby, M. Ashrafian-Bonab, L. Farrell et al., "Distribution of killer cell immunoglobulin-like receptors (KIR) and their HLA-C ligands in two Iranian populations," *Immunogenetics*, vol. 62, no. 2, pp. 65–73, 2010.

[17] S. E. Hiby, R. Apps, A. M. Sharkey et al., "Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2," *Journal of Clinical Investigation*, vol. 120, no. 11, pp. 4102–4110, 2010.

[18] Z. Lu, B. Zhang, S. Chen et al., "Association of KIR genotypes and haplotypes with susceptibility to chronic hepatitis B virus infection in Chinese Han population," *Cellular & Molecular Immunology*, vol. 5, no. 6, pp. 457–463, 2008.

[19] K. Cheent and S. I. Khakoo, "Natural killer cells: integrating diversity with function," *Immunology*, vol. 126, no. 4, pp. 449–457, 2009.

[20] A. Kikuchi-Maki, T. L. Catina, and K. S. Campbell, "Cutting edge: KIR2DL4 transduces signals into human NK cells through association with the Fc receptor γ protein," *Journal of Immunology*, vol. 174, no. 7, pp. 3859–3863, 2005.

[21] S. I. Khakoo, R. Rajalingam, B. P. Shum et al., "Rapid evolution of NK cell receptor systems demonstrated by comparison of chimpanzees and humans," *Immunity*, vol. 12, no. 6, pp. 687–698, 2000.

[22] M. Colonna, G. Borsellino, M. Falco, G. B. Ferrara, and J. L. Strominger, "HLA-C is the inhibitory ligand that determines dominant resistance to lysis by NK1- and NK2-specific natural killer cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 24, pp. 12000–12004, 1993.

[23] N. Wagtmann, S. Rajagopalan, C. C. Winter, M. Peruzzi, and E. O. Long, "Killer cell inhibitory receptors specific for HLA-C and HLA-B identified by direct binding and by functional transfer," *Immunity*, vol. 3, no. 6, pp. 801–809, 1995.

[24] A. K. Moesta, P. J. Norman, M. Yawata, N. Yawata, M. Gleimer, and P. Parham, "Synergistic polymorphism at two positions distal to the ligand-binding site makes KIR2DL2 a stronger receptor for HLA-C Than KIR2DL3," *Journal of Immunology*, vol. 180, no. 6, pp. 3969–3979, 2008.

[25] C. C. Winter, J. E. Gumperz, P. Parham, E. O. Long, and N. Wagtmann, "Direct binding and functional transfer of NK cell inhibitory receptors reveal novel patterns of HLA-C allotype recognition," *Journal of Immunology*, vol. 161, no. 2, pp. 571–577, 1998.

[26] J. E. Gumperz, V. Litwin, J. H. Phillips, L. L. Lanier, and P. Parham, "The Bw4 public epitope of HLA-B molecules confers reactivity with natural killer cell clones that express NKB1, a putative HLA receptor," *Journal of Experimental Medicine*, vol. 181, no. 3, pp. 1133–1144, 1995.

[27] D. Pende, R. Biassoni, C. Cantoni et al., "The natural killer cell receptor specific for HLA-A allotypes: a novel member of the p58/p70 family of inhibitory receptors that is characterized by three immunoglobulin-like domains and is expressed as a 140-kD disulphide- linked dimer," *Journal of Experimental Medicine*, vol. 184, no. 2, pp. 505–518, 1996.

[28] C. A. Stewart, F. Laugier-Anfossi, F. Vély et al., "Recognition of peptide-MHC class I complexes by activating killer immunoglobulin-like receptors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 37, pp. 13224–13229, 2005.

[29] A. K. Moesta, T. Graef, L. Abi-Rached, A. M. O. Aguilar, L. A. Guethlein, and P. Parham, "Humans differ from other hominids in lacking an activating NK cell receptor that recognizes the C1 epitope of MHC class I," *Journal of Immunology*, vol. 185, no. 7, pp. 4233–4237, 2010.

[30] D. H. Raulet and R. E. Vance, "Self-tolerance of natural killer cells," *Nature Reviews Immunology*, vol. 6, no. 7, pp. 520–531, 2006.

[31] S. Kim, J. Poursine-Laurent, S. M. Truscott et al., "Licensing of natural killer cells by host major histocompatibility complex class I molecules," *Nature*, vol. 436, no. 7051, pp. 709–713, 2005.

[32] H. R. MacDonald, "NK cell tolerance: revisiting the central dogma," *Nature Immunology*, vol. 6, no. 9, pp. 868–869, 2005.

[33] G. Alter et al., "Reduced frequencies of NKp30+NKp46+, CD161+ and NKG2D+ NK cells in acute HCV infection may predict viral clearance," *Journal of Hepatology*, 2010. In press.

[34] B. Amadei, S. Urbani, A. Cazaly et al., "Activation of natural killer cells during acute infection with hepatitis C virus," *Gastroenterology*, vol. 138, no. 4, pp. 1536–1545, 2010.

[35] S. Pelletier, C. Drouin, N. Bédard, S. I. Khakoo, J. Bruneau, and N. H. Shoukry, "Increased degranulation of natural killer cells during acute HCV correlates with the magnitude of virus-specific T cell responses," *Journal of Hepatology*, vol. 53, no. 5, pp. 805–816, 2010.

[36] C. Morishima, D. M. Paschal, C. C. Wang et al., "Decreased NK cell frequency in chronic hepatitis C does not affect ex vivo cytolytic killing," *Hepatology*, vol. 43, no. 3, pp. 573–580, 2006.

[37] J. Corado, F. Toro, H. Rivera, N. E. Bianco, L. Deibis, and J. B. De Sanctis, "Impairment of natural killer (NK) cytotoxic activity in hepatitis C virus (HCV) infection," *Clinical and Experimental Immunology*, vol. 109, no. 3, pp. 451–457, 1997.

[38] G. Pár, D. Rukavina, E. R. Podack et al., "Decrease in CD3-negative-CD8dim$^+$ and Vδ2/Vγ9 TcR + peripheral blood lymphocyte counts, low perforin expression and the impairment of natural killer cell activity is associated with chronic hepatitis C virus infection," *Journal of Hepatology*, vol. 37, no. 4, pp. 514–522, 2002.

[39] M. S. Bonavita, A. Franco, M. Paroli et al., "Normalization of depressed natural killer activity after interferon-α therapy is associated with a low frequency of relapse in patients with chronic hepatitis C," *International Journal of Tissue Reactions*, vol. 15, no. 1, pp. 11–16, 1993.

[40] S. I. Khakoo, C. L. Thio, M. P. Martin et al., "HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection," *Science*, vol. 305, no. 5685, pp. 872–874, 2004.

[41] V. Romero, J. Azocar, J. Zúñiga et al., "Interaction of NK inhibitory receptor genes with HLA-C and MHC class II alleles in Hepatitis C virus infection outcome," *Molecular Immunology*, vol. 45, no. 9, pp. 2429–2436, 2008.

[42] L. Fadda, G. Borhis, P. Ahmed et al., "Peptide antagonism as a mechanism for NK cell activation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 22, pp. 10160–10165, 2010.

[43] G. Ahlenstiel, M. P. Martin, X. Gao, M. Carrington, and B. Rehermann, "Distinct KIR/HLA compound genotypes affect the kinetics of human antiviral natural killer cell responses," *Journal of Clinical Investigation*, vol. 118, no. 3, pp. 1017–1026, 2008.

[44] S. Knapp, U. Warshow, D. Hegazy et al., "Consistent beneficial effects of killer cell immunoglobulin-like receptor 2dl3 and group 1 human leukocyte antigen-c following exposure to hepatitis c virus," *Hepatology*, vol. 51, no. 4, pp. 1168–1175, 2010.

[45] J. R. Vidal-Castiñeira, A. López-Vázquez, R. Díaz-Peña et al., "Effect of killer immunoglobulin-like receptors in the response to combined treatment in patients with chronic hepatitis C virus infection," *Journal of Virology*, vol. 84, no. 1, pp. 475–481, 2010.

[46] A. López-Vázquez, L. Rodrigo, J. Martínez-Borra et al., "Protective effect of the HLA-Bw4I80 epitope and the killer cell immunoglobulin-like receptor 3DS1 gene against the development of hepatocellular carcinoma in patients with hepatitis C virus infection," *Journal of Infectious Diseases*, vol. 192, no. 1, pp. 162–165, 2005.

[47] J. Zúñiga, V. Romero, J. Azocar et al., "Protective KIR-HLA interactions for HCV infection in intravenous drug users," *Molecular Immunology*, vol. 46, no. 13, pp. 2723–2727, 2009.

[48] V. L. Carneiro, D. C. Lemaire, M. T. Bendicho et al., "Natural killer cell receptor and HLA-C gene polymorphisms among patients with hepatitis C: a comparison between sustained virological responders and non-responders," *Liver International*, vol. 30, no. 4, pp. 567–573, 2010.

[49] A. Rauch, R. Laird, E. McKinnon et al., "Influence of inhibitory killer immunoglobulin-like receptors and their HLA-C ligands on resolving hepatitis C virus infection," *Tissue Antigens*, vol. 69, no. 1, pp. 237–240, 2007.

[50] B. Oliviero, S. Varchetta, E. Paudice et al., "Natural killer cell functional dichotomy in chronic hepatitis B and chronic hepatitis C virus infections," *Gastroenterology*, vol. 137, no. 3, pp. 1151–1160.e7, 2009.

[51] R. J. Harrison, A. Ettorre, A.-M. Little, and S. I. Khakoo, "Association of NKG2A with treatment for chronic hepatitis C virus infection," *Clinical and Experimental Immunology*, vol. 161, no. 2, pp. 306–314, 2010.

[52] G. Ahlenstiel, R. H. Titerence, C. Koh et al., "Natural killer cells are polarized toward cytotoxicity in chronic hepatitis C in an interferon-alfa-dependent manner," *Gastroenterology*, vol. 138, no. 1, pp. 325–335.e2, 2010.

[53] World Health Organization, "Fact Sheet 204," 2008, http://www.who.int/mediacentre/factsheets/fs204/en/.

[54] B. Custer, S. D. Sullivan, T. K. Hazlet, U. Iloeje, D. L. Veenstra, and K. V. Kowdley, "Global epidemiology of hepatitis B virus," *Journal of clinical gastroenterology*, vol. 38, no. 10, pp. S158–S168, 2004.

[55] X. Gao, Y. Jiao, L. Wang et al., "Inhibitory KIR and specific HLA-C gene combinations confer susceptibility to or protection against chronic hepatitis B," *Clinical Immunology*, vol. 137, no. 1, pp. 139–146, 2010.

[56] M. P. Martin, X. Gao, J. H. Lee et al., "Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS," *Nature Genetics*, vol. 31, no. 4, pp. 429–434, 2002.

[57] Y. Qi, M. P. Martin, X. Gao et al., "KIR/HLA pleiotropism: protection against both HIV and opportunistic infections.," *PLoS Pathogens*, vol. 2, no. 8, article e79, 2006.

[58] J. D. Barbour, U. Sriram, S. J. Caillier, J. A. Levy, F. M. Hecht, and J. R. Oksenberg, "Synergy or independence? Deciphering the interaction of HLA Class I and NK cell KIR alleles in early HIV-1 disease progression.," *PLoS pathogens*, vol. 3, no. 4, article e43, 2007.

[59] G. B. Cohen, R. T. Gandhi, D. M. Davis et al., "The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells," *Immunity*, vol. 10, no. 6, pp. 661–671, 1999.

[60] R. Biassoni, A. Pessino, A. Malaspina et al., "Role of amino acid position 70 in the binding affinity of p50.1 and p58.1 receptors for HLA-Cw4 molecules," *European Journal of Immunology*, vol. 27, no. 12, pp. 3095–3099, 1997.

[61] G. Katz, G. Markel, S. Mizrahi, T. I. Arnon, and O. Mandelboim, "Recognition of HLA-Cw4 but not HLA-Cw6 by the NK cell receptor killer cell Ig-like receptor two-domain short tail number 4," *Journal of Immunology*, vol. 166, no. 12, pp. 7260–7267, 2001.

[62] M. S. Malnati, M. Peruzzi, K. C. Parker et al., "Peptide specificity in the recognition of MHC class I by natural killer cell clones," *Science*, vol. 267, no. 5200, pp. 1016–1018, 1995.

[63] S. Gaudieri, D. DeSantis, E. McKinnon et al., "Killer immunoglobulin-like receptors and HLA act both independently and synergistically to modify HIV disease progression," *Genes and Immunity*, vol. 6, no. 8, pp. 683–690, 2005.

[64] S. Boulet, S. Sharafi, N. Simic et al., "Increased proportion of KIR3DS1 homozygotes in HIV-exposed uninfected individuals," *AIDS*, vol. 22, no. 5, pp. 595–599, 2008.

[65] G. Alter, M. P. Martin, N. Teigen et al., "Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes," *Journal of Experimental Medicine*, vol. 204, no. 12, pp. 3027–3036, 2007.

[66] B. R. Long, L. C. Ndhlovu, J. R. Oksenberg et al., "Conferral of enhanced natural killer cell function by KIR3DS1 in early human immunodeficiency virus type 1 infection," *Journal of Virology*, vol. 82, no. 10, pp. 4785–4792, 2008.

[67] R. Thomas, E. Yamada, G. Alter et al., "Novel KIR3DL1 alleles and their expression levels on NK cells: convergent evolution of KIR3DL1 phenotype variation?" *Journal of Immunology*, vol. 180, no. 10, pp. 6743–6750, 2008.

[68] M. P. Martin, Y. Qi, X. Gao et al., "Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1," *Nature Genetics*, vol. 39, no. 6, pp. 733–740, 2007.

[69] G. M. O'Connor, K. J. Guinan, R. T. Cunningham, D. Middleton, P. Parham, and C. M. Gardiner, "Functional polymorphism of the KIR3DL1/S1 receptor on human NK cells," *Journal of Immunology*, vol. 178, no. 1, pp. 235–241, 2007.

[70] S. Rajagopalan, Y. T. Bryceson, S. P. Kuppusamy et al., "Activation of NK cells by an endocytosed receptor for soluble HLA-G," *PLoS Biology*, vol. 4, no. 1, article no. e9, 2006.

[71] S. Boulet, R. Song, P. Kamya et al., "HIV protective KIR3DL1 and HLA-B genotypes influence NK cell function following stimulation with HLA-devoid cells," *Journal of Immunology*, vol. 184, no. 4, pp. 2057–2064, 2010.

[72] J. Fellay, K. V. Shianna, D. Ge et al., "A whole-genome association study of major determinants for host control of HIV-1," *Science*, vol. 317, no. 5840, pp. 944–947, 2007.

[73] F. Pereyra, X. Jia, P. J. McLaren, A. Telenti, P. I. W. De Bakker, and B. D. Walker, "The major genetic determinants of HIV-1 control affect HLA class I peptide presentation," *Science*, vol. 330, no. 6010, pp. 1551–1557, 2010.

[74] A. T. Makadzange, G. Gillespie, T. Dong et al., "Characterization of an HLA-C-restricted CTL response in chronic HIV infection," *European Journal of Immunology*, vol. 40, no. 4, pp. 1036–1041, 2010.

[75] W. Jennes, S. Verheyden, C. Demanet et al., "Cutting edge: resistance to HIV-1 infection among African female sex workers is associated with inhibitory KIR in the absence of their HLA ligands," *Journal of Immunology*, vol. 177, no. 10, pp. 6588–6592, 2006.

[76] R. Thomas, R. Apps, Y. Qi et al., "HLA-C cell surface expression and control of HIV/AIDS correlate with a variant upstream of HLA-C," *Nature Genetics*, vol. 41, no. 12, pp. 1290–1294, 2009.

[77] C. A. Biron, K. S. Byron, and J. L. Sullivan, "Severe herpesvirus infections in an adolescent without natural killer cells," *New England Journal of Medicine*, vol. 320, no. 26, pp. 1731–1735, 1989.

[78] M. Cook, D. Briggs, C. Craddock et al., "Donor KIR genotype has a major influence on the rate of cytomegalovirus reactivation following T-cell replete stem cell transplantation," *Blood*, vol. 107, no. 3, pp. 1230–1232, 2006.

[79] C. Chen, M. Busson, V. Rocha et al., "Activating KIR genes are associated with CMV reactivation and survival after non-T-cell depleted HLA-identical sibling bone marrow transplantation for malignant disorders," *Bone Marrow Transplantation*, vol. 38, no. 6, pp. 437–444, 2006.

[80] J. A. Zaia, J. Y. Sun, G. M. Gallez-Hawkins et al., "The effect of single and combined activating killer immunoglobulin-like receptor genotypes on cytomegalovirus infection and immunity after hematopoietic cell transplantation," *Biology of Blood and Marrow Transplantation*, vol. 15, no. 3, pp. 315–325, 2009.

[81] V. R. Bonagura, Z. Du, E. Ashouri et al., "Activating killer cell immunoglobulin-like receptors 3DS1 and 2DS1 protect against developing the severe form of recurrent respiratory papillomatosis," *Human Immunology*, vol. 71, no. 2, pp. 212–219, 2010.

[82] M. Carrington, S. Wang, M. P. Martin et al., "Hierarchy of resistance to cervical neoplasia mediated by combinations of killer immunoglobulin-like receptor and human leukocyte antigen loci," *Journal of Experimental Medicine*, vol. 201, no. 7, pp. 1069–1075, 2005.

[83] E. Estefanía, N. Gómez-Lozano, F. Portero et al., "Influence of KIR gene diversity on the course of HSV-1 infection: resistance

to the disease is associated with the absence of KIR2DL2 and KIR2DS2," *Tissue Antigens*, vol. 70, no. 1, pp. 34–41, 2007.

[84] D. S. Korbel, O. C. Finney, and E. M. Riley, "Natural killer cells and innate immunity to protozoan pathogens," *International Journal for Parasitology*, vol. 34, no. 13-14, pp. 1517–1528, 2004.

[85] K. Artavanis-Tsakonas, K. Eleme, K. L. McQueen et al., "Activation of a subset of human NK cells upon contact with Plasmodium falciparum-infected erythrocytes," *Journal of Immunology*, vol. 171, no. 10, pp. 5396–5405, 2003.

[86] M. Baratin, S. Roetynck, B. Pouvelle et al., "Dissection of the role of PfEMP1 and ICAM-1 in the sensing of Plasmodium falciparum-infected erythrocytes by natural killer cells," *PLoS One*, vol. 2, no. 2, article no. e228, 2007.

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Review Article

The Structural Basis of Ligand Recognition by Natural Killer Cell Receptors

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Natural killer cells are a group of lymphocytes which function as tightly controlled surveillance operatives which identify transformed cells through a discrete balance of activating and inhibitory receptors ultimately leading to the destruction of incongruent cells. The understanding of this finely tuned balancing act has been aided by the high-resolution structure determination of activating and inhibitory receptors both alone and in complex with their ligands. This paper collates these structural studies detailing the aspects which directly relate to the natural killer cell function and serves to inform both the specialized structural biologist reader and a more general immunology audience.

1. Introduction

The functional outcome of Natural Killer (NK) cells is determined by integrating both activating and inhibitory signals resulting in a highly controlled response which mediates cytotoxicity against transformed cells and, in addition, the release of cytokines critical to the immune response. At the center of this balance resides a group of receptors that allow the relay of intracellular signaling via intrinsic or associated cytoplasmic molecular motifs for various kinases or phosphatases [1–4]. The list of identified NK cell activating and inhibitory receptors currently exceeds twenty. In humans, the activating NK receptors include CD16, the short-tail members of killer immunoglobulin-like receptors (KIRs), CD94/NKG2C, NKG2D, 2B4, NKp30, NKp44, and NKp46 (Figure 1). They signal through association with either DAP10 which allows PI3-kinase activation or DAP12, CD3ζ, or FcεRIγ, which all contain immunotyrosine-based activation motifs (ITAM). In addition, adhesion molecules, such as LFA-1 and DNAM-1, are also important for the lytic function of NK cells, but they do not associate directly with the known ITAM-containing molecules [5]. The inhibitory NK receptors include the long-tail members of the KIR family, CD94/NKG2A, leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1), and killer cell lectin-like receptor subfamily G member 1 (KLRG-1) which signal via cytoplasmic immunotyrosine-based inhibitory motifs (ITIM). From a structural perspective, all known NK receptors adopt either an immunoglobulin- (Ig-) like or a C-type lectin-like receptor fold with both folds present in the activating and inhibitory receptor families. In the last decade, a large amount of structural information for many of these receptors has become available, and this review focuses on the structures of human NK cell receptors and their ligand recognition (Table 1).

2. Immunoglobulin-Like NK Receptors

2.1. Structure of KIR. Shortly after the identification of inhibitory NK cell receptors and their MHC ligands, questions regarding the mechanisms of ligand recognition were raised. Prior to the NK cell receptor ligand findings, T cell receptors were the only MHC binding molecules. Characterization of the TCR-MHC recognition mechanism was a central tenet in molecular immunology. The fundamental principle of TCR-MHC recognition is based on a number of factors which include the presentation of peptides by a MHC molecule and the polymorphic MHC residues which

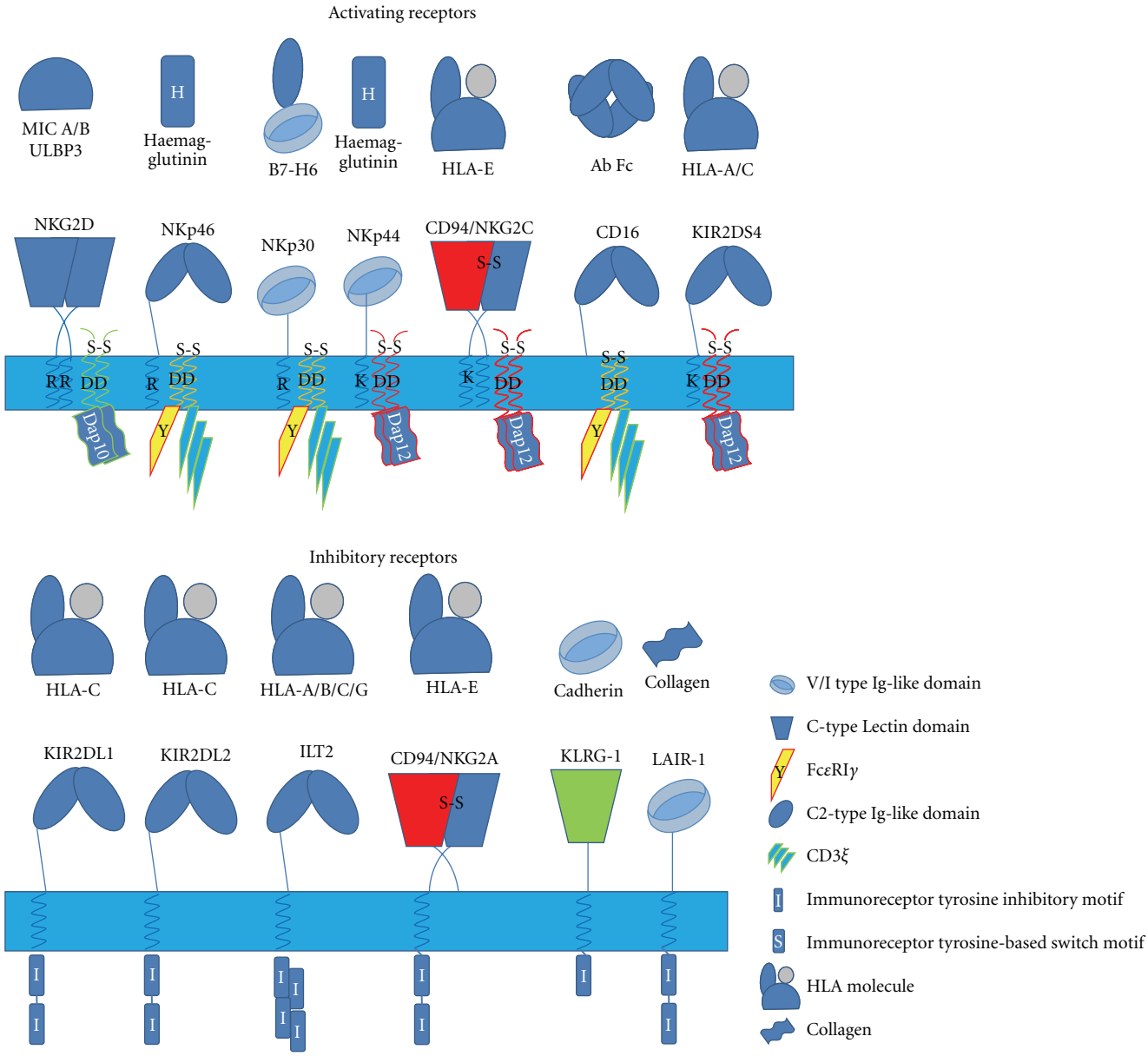

Figure 1: Overview of the structurally characterized activating and inhibitory human NK receptors and their ligands. The upper panel displays the chief activating receptors found on human NK cells including the homodimer NKG2D, the natural cytotoxicity receptors, NKp46, NKp44, and NKp30, the c-type lectin heterodimer CD94 : NKG2C, the Fc receptor CD16, and also the KIR activating receptors. The associated cytoplasmic signaling molecules for these activating receptors which include DAP12, DAP10, and CD3ζ/FcεRIγ and the specific transmembrane residues which facilitate the interaction are also shown. The lower panel illustrates the structurally characterized inhibitory receptors found on NK cells. These include the KIR receptors KIR 2DL1 and KIR 2DL2 which have been characterized in complex with peptide bound MHC class I molecules, the heterodimeric CD94 : NKG2A which binds to peptide bound HLA-E. The C-type lectin receptor KLRG-1 which binds to cadherin and the NK cell receptor LAIR-1 molecule which binds to collagen are also shown. The known ligands for both the activating and inhibitory receptors are illustrated above their respective NK cell receptor.

together interact with the TCR. In addition, the TCR uses complementarity determining regions (CDR) to recognize MHC molecules, in a similar manner to that seen with antibody ligand interactions. These CDR regions are the result of gene rearrangement and recombination, thus essentially allowing an unlimited number of TCR specificities. This diverse receptor repertoire is necessary to recognize variable pathogenic peptides and the presenting polymorphic MHC molecules which altogether enable exquisite TCR-MHC allele specificities.

The identification of NK receptors which could bind to MHC molecules immediately challenged our understanding of MHC recognition. While the potential T cell receptor repertoire can be greater than 10^6 molecules, NK receptors

Table 1: List of human NK receptor structures.

Structure	Resolution (Å)	PDB entry	Reference
KIR2DL1	1.7	1NKR	Fan et al. [6]
KIR2DL2	2.9	2DLI	Snyder et al. [10]
		2DL2	
KIR2DL3	3.0	1B6U	Maenaka et al. [8]
KIR2DS2	2.3	1M4K	Saulquin et al. [9]
KIR2DS4	2.5	3H8N	Graef et al. [7]
KIR2DL2/HLA-Cw3	3.0	1EFX	Boyington et al. [11]
KIR2DL1/HLA-Cw4	2.8	1IM9	Fan et al. [12]
NKp46	2.2	1P6F	Foster et al. [13]
	1.9	1OLL	Ponassi et al. [14]
NKp44	2.2	1HKF	Cantoni et al. [15]
NKp30	1.8	3NOI	Joyce et al. [16]
LAIR-1	1.8	3KGR	Brondijk et al., 2010
CD94	2.6	1B6E	Boyington et al. [17]
CD94/NKG2A	2.5	3BDW	Sullivan et al. [18]
CD94/NKG2A/HLA-E	2.5	3CDG	Petrie et al. [19]
	3.4	3CII	Kaiser et al. [20]
NKG2D	1.95	1HQ8	Wolan et al. [21]
NKG2D/MICA	2.7	1HYR	Li et al. [22]
NKG2D/ULBP3	2.6	1KCG	Radaev et al. [23]
NKG2D/Rae-1β	3.5	1JSK	Li et al. [24]
KLRG1/E-cadherin	1.8	3FF7	Li et al. [25]
DAP12/NKG2C	NMR	2L35	Call et al. [26]

are germ-line encoded and there are less than a dozen KIR molecules in a given individual. A number of questions arose such as how can such a small number of KIRs recognize a much larger number of peptide-MHC molecules? While the KIR genes are also members of the Ig super family, could they form TCR-like structures and recognize MHC molecules in a similar fashion to the TCRs even though the putative CDRs are nonvariable?

Broadly speaking, there are overall large differences between the size and structures of TCR molecules compared to the KIR molecules. TCR molecules are made up of a heterodimer of an alpha and beta chain each constituting ~200 amino acids in length and peptide-MHC recognition is dictated by both the alpha and beta chains while in contrast KIR molecules are monomeric consisting of ~200 amino acids in length. To date, there are five crystal structures available for KIR family members, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DS2, and KIR2DS4 [6–10]. All structures display essentially the same fold with two C2-type Ig-like domains, each made up of a β-sandwich with strands ABE packed against C′CFGA′ (Figure 2; note that in contrast to C2-type Ig-domains, a C1-type Ig-like domain is made up of ABED strands stacking against CFG strands). The two Ig-like domains, named D1 and D2, exhibit a tilted side by side arrangement with a major hinge region, which results in a substantial surface buried area between the D1 and D2 domains. The hinge angle observed in the structure of KIR2DL1 was about 65°, substantially smaller than those in the other four structures, which were about 80°, which indicated that the hinge angle might be important for ligand binding. However, the hinge angle of KIR2DL1 in the presence of bound HLA molecule was found to be similar to that of KIR2DL2, suggesting that the smaller hinge angle observed in the ligand-free structure of KIR 2DL1 is likely influenced by crystal packing. Many of the hinge residues are conserved among members of the KIR family, including the three domain KIRs, suggesting they all share the same relative domain arrangement. It is worth noting, however, that there is no structure available at present for any member of the KIR3D receptors which differ from the KIR2D molecules by the presence of an additional D0 domain.

2.2. KIR Recognition of MHC Molecules. Understanding KIR recognition of MHC molecules through the mapping of mutational data on the KIR structure suggested that critical MHC contact regions were not at the tip of the KIR D1 domain as is the case with TCR receptor binding, rather they were located near the receptor hinge region [6, 10]. Furthermore, while KIRs were known to recognize degenerate MHC ligands, the ligand recognition was not entirely promiscuous, but in fact rather specific. That is, KIR2DL2 has C1-type specificity (KIR molecule contains Lys-44 and binds to Asn-80 residue in HLA-molecule) recognizing HLA-Cw1, 3, 7, and 8. In contrast, KIR2DL1 has C2-type specificity (KIR molecule contains Met-44 and binds to Lys-80 residue in HLA molecule) binding to the ligands HLA-Cw2, 4, 5, 6, and 15. How can MHC molecules with their polymorphic heavy chains which are necessary for presenting variable

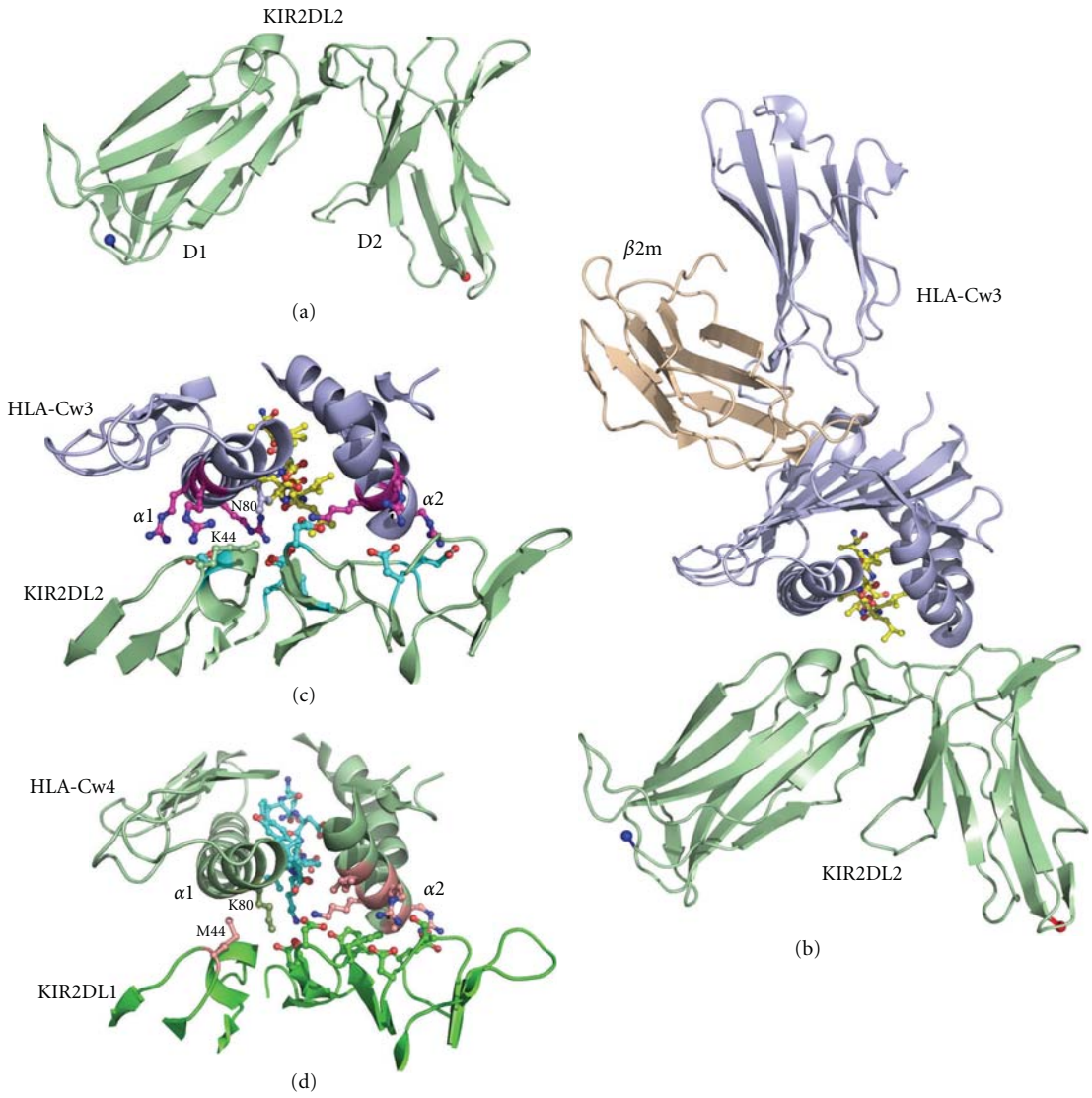

Figure 2: Structure of inhibitory KIR molecules. (a) Structure of KIR2DL2 which is made up of two C2-like domains is shown in cartoon representation with the N-terminus and C-terminus described by a blue and red sphere, respectively. (b) Structure of KIR2DL2 in complex with the MHC class I molecule HLA-Cw3 and β2m shown in cartoon representation with bound peptide shown in ball and stick representation with yellow carbon atoms. (c) Closeup of the HLA-Cw3 : KIR2DL2 interface with interacting residues displayed in ball and stick representation. The bound peptide is shown with yellow colored carbon atoms while the interacting residues from HLA-Cw3 are shown in purple and those from the KIR molecule are shown in off-white coloring. The critical residues for HLA-KIR specificity, K44 from KIR 2DL2 and N80 from HLA Cw3 are shown in green and light-blue, respectively. (d) Closeup of the HLA-Cw4 : KIR2DL1 interface with interacting residues displayed in ball and stick representation. The bound peptide is shown with white carbon atoms while the interacting residues from HLA-Cw4 have green carbon atoms and those from KIR 2DL1 have yellow carbon atoms. The critical residues for HLA-KIR specificity, M44 from KIR 2DL1, and K80 from HLA Cw4 are shown in dark green and light green, respectively.

peptides, and whose recognition requires variable regions of TCR, serve as ligands to nonvariable KIR receptors? These principle defining questions prompted an intense effort to resolve the structure of KIR in complex with HLA ligands.

The first KIR-MHC complex structure was determined between KIR2DL2 and HLA-Cw3 bound to a nonamer self-peptide from human importin-α1 [11]. Subsequently, the structure of KIR2DL1 in complex with HLA-Cw4 was also determined [12]. Since the two KIR receptors recognize different class I MHC allotypes, the structure solution of both KIR-MHC complexes provided a rather complete and complementary structural view of both the overall KIR-MHC recognition and the specific interactions critical to their class I MHC specificities. The overall KIR binding mode used for HLA recognition is very similar between the two complexes. Both KIR2DL1 and KIR2DL2 bind to the peptide-binding groove of the MHC molecules using the receptor D1 and D2 domains which interact with the α1- and

α2-helices of the MHC heavy chain, respectively (Figures 2(c) and 2(d)). Although many detailed interface interactions differ between the two KIR-MHC complexes, both KIRs recognize their MHC ligands through primarily salt bridges and charge complementarity rather than hydrophobic interactions and shape complementarity. KIR receptors contact primarily nonpolymorphic HLA residues on both the α1- and α2-helices. The molecular mechanism of the C1- and C2 allotype specificities of KIR also became apparent. KIR2DL2 can be switched to recognize C2 ligands by just one amino acid mutation at residue 44 [27]. Similarly, the C1 and C2 type class I ligand engagement appeared to be controlled by residue 80 on the MHC heavy chain [28]. The structures suggest that this specificity switch is due to primarily the compatibility of one hydrogen bond between Lys 44 of KIR 2DL2 and Asn 80 of HLA-Cw3 [11].

The overall KIR docking orientation on the MHC molecule is very similar to that of TCR with the D1 and D2 domains of KIRs occupying equivalent positions to the Vα and Vβ of a TCR. However, KIR receptor binding to the peptide is altered in comparison to TCR binding. Typically peptides which are bound to the MHC class I molecules are 8–10 amino acids in size and they are numbered from N-terminus to C-terminus with the first peptide amino acid named P1 and so on. TCR binding interactions with the peptide are primarily centered on the P4-P5 amino acids and are highly specific while KIR binding is shifted towards the C-terminus of the bound peptide interacting with the P7 and P8 residues of the peptide. This results in a mutually exclusive region of peptide binding between KIR and TCR but also some level of overlap. This partial overlap between KIR and TCR is potentially important to avoid conflicting simultaneous recognition by both the activating T and inhibitory NK cell receptors. The nonoverlapping peptide regions used in KIR and TCR binding mechanisms allows a TCR to evolve and mature against the variable parts of presented peptides and also the polymorphic MHC residues while KIR maintains a dual recognition mechanism of both the nonpolymorphic MHC residues and bound self-peptides via its specific binding mechanism ultimately resulting in inhibition of NK cell activation pathways.

The peptide sensitivity of KIR-MHC recognition remains an important issue. While the structures show that KIR receptors either directly or through water molecules interact with the MHC bound peptide, the KIR-peptide interactions only serve to constrain the type of residues placed at the P7 and P8 position of the peptide instead of formally defining specific contacts. In the case of KIR2DL2, the receptor and peptide contact requires a hydrophobic residue at the P7 and the presence of a small amino acid, such as an Ala or Ser residue at the P8 position [11]. Whether such constraints contribute to KIR differentiation of self versus pathogenic peptides remains an open question. Since the peptide positions recognized by KIR are not involved in TCR binding, it is thus likely that a self-peptide presenting an MHC ligand of KIR can also present pathogenic peptides for TCR. In other words, MHC molecules can evolve simultaneously with abilities both to rapidly mutate their polymorphic regions to match and present pathogenic antigens for T cell-mediated immunity and also to maintain the nonvariant regions for self peptide binding and recognition by KIR for NK cell mediated immune protection.

2.3. Activating Killer Ig-Like Receptors. While the inhibitory group of KIR molecules has been extensively studied, the function and ligands of the activating KIR molecules are less well understood. Activating KIR molecules occur less commonly than the inhibitory KIR receptors and are comprised of the two domain KIR2DS family and the three domain KIR3DS family. KIR2DS1 has similar binding specificity to KIR2DL1 in that both bind to HLA-C2 but KIR2DS1 has a much lower affinity for this receptor. Illustrating the lack of understanding of these receptors is the fact that the ligands for KIR2DS2, KIR2DS3, KIR2DS5, and KIR3DS1 are currently unknown.

Sequence comparison between activating and inhibitory ligands indicates that the activating and inhibitory molecules can be shockingly close in sequence identity to each other but the most minimal changes still result in differences in ligand binding specificity and affinity. One example is the case of KIR2DS2 in which the ligand specificity is unknown while this activating molecule has been structurally characterized [9]. The sequence difference between KIR2DS2 and KIR2DL3 is only two amino acid changes with Tyr45 and Glu35 found in KIR2DS2 as opposed to Phe45 and Gln35 in KIR2DL3. The ligand for KIR2DL3 is known to be HLA-C molecules but KIR2DS2 has undetectable binding affinity for these molecules. KIR2DS2 differs in sequence from KIR2DL2 by four amino acids as follows, P16R, Y45F, R148C, and T200I. The F45Y change in KIR2DS2 does not lead to any steric clashes based on modeling of bound HLA molecule but it will clearly disrupt extensive hydrophobic interactions which are seen in the 2DL2 structure perhaps explaining the absence of HLA-C binding (Figures 3(b) and 3(f)).

A second activating KIR, KIR2DS4, has recently been characterized as an HLA class I receptor which binds specifically to subsets of $C1^+$ and $C2^+$ HLA-C and also to HLA-A*11 molecules [7]. The presence of activating KIR molecules appears to occur late in evolution and only KIR2DS4 has a homologue in any other species [29]. The structure of this activating KIR molecule KIR2DS4 has also been determined [7]; (Figure 3). Overall, the structure of KIR2DS4 is very similar to other KIR molecules as judged by very low rmsd values (KIR2DL1 bound structure: rmsd 1.04 Å; KIR2DL2 bound structure: rmsd 1.12 Å; KIR2DS2: rmsd 2.37 Å). KIR2DS4 D1/D2 domains form an angle of 69° which is similar to the unbound KIR2DL1 structure (66°) but contrasts with the KIR2DL2 (81°) and KIR2DL3 (78°) structures. Further analysis of the hinge region residues illustrates a core hydrophobic region which is made up of 13 amino acids and is very similar to the inhibitory KIR molecules. Of these residues, only residue 102 shows some variability amongst the KIR family, and this has been shown to lead to changes in HLA specificity implying the hinge region hydrophobic core can indirectly impact ligand specificity and binding. Comparison of KIR2DS4 with other KIR molecules indicates that the main structural changes include the altered orientation of residues 43–45 away from

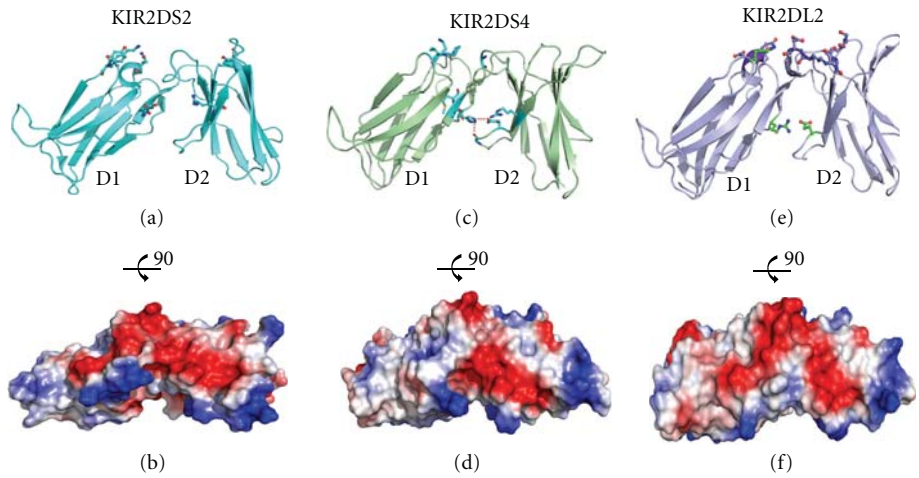

Figure 3: Structure of activating KIR receptors. (a) KIR2DS2 shown in cartoon representation with residues likely important for ligand binding and also the hinge region residues shown in ball and stick representation. (b) Charged surface representation of the membrane distal face (binding face) of KIR2DS2. (c) KIR2DS4 shown in cartoon representation with ligand binding and hinge region residues shown in ball and stick representation. (d) Charged surface representation of the membrane distal face (binding face) of KIR2DS4. (e) For comparative purposes, the inhibitory receptor KIR2DL2 is shown in cartoon representation with ligand binding and hinge region residues shown in ball and stick representation. (f) Charged surface representation of the membrane distal face (binding face) of the inhibitory receptor KIR2DL2.

the ligand binding area which results in the critical Lys44 of inhibitory KIRs lying ~2.5 Å away from the location observed in KIR2DL2/3. A second critical region is the Pro71-Val72 region which differs from the KIR2DL2 Gln71-Asp72 residues and is likely one of the main specificity components for KIR2DS4.

The presence of activating receptors with such close ligand specificity to the inhibitory KIR molecules is somewhat perplexing, but analysis of the presence of these genes in different populations indicates that KIR2DS4 may play a role in EBV control within Southeast Asian populations [30]. There is also support for the role of activating receptors in the control of cytomegalovirus infection following kidney transplantation [31]. The presence of paired or balancing KIR receptors may exist to prevent viral mutations which would result in uncontrolled infections. An additional role of activating KIR receptors in embryo implantation and the initial stages of pregnancy has been illuminated by Moffett and coworkers [32]. Their studies indicate that the presence of the activating KIR2DS1 receptor may influence embryo implantation and prevent reproductive failure.

2.4. Natural Cytotoxicity Receptors. The natural cytotoxicity receptors have been identified as the effector molecules responsible for the majority of NK driven cytotoxicity against tumor cells or virally infected cells with blocking of these receptors resulting in significantly decreased NK cell killing [33]. The NCRs are made up of three Ig-like proteins named NKp46, NKp30, and NKp44. NKp46 and NKp30 are constitutively expressed on NK cells while NKp44 is expressed upon IL-2 driven activation of NK cells. To date, all three proteins have been structurally characterized (Figure 4) but some controversy still surrounds their ligands due to the absence of ligand bound structures.

NKp46 was first identified by Moretta and coworkers in 1997 as a cell surface receptor expressed on freshly isolated and activated NK cells and which plays a pivotal role in natural cytotoxicity and targeting of transformed cells [34]. NKp46 is critical to NK cell activation in response to Newcastle disease virus [35], primary tumor melanocytes [36], urothelial cancers [37], medulloblastoma [38], myeloma cancer [39], filovirus infected dendritic cells [40], Herpes simplex virus [41], vaccinia virus [42], influenza virus, parainfluenza and sendai viruses [43], and the response to mycobacterium tuberculosis infected monocytes [44]. NKp46 is a type I membrane protein made up of two N-terminal Ig-like extracellular domains, a 40 amino acid linker region, single transmembrane domain, and a short highly charged 25 amino acid cytoplasmic region. NKp46 contains three glycosylation sites, two which are located in the 40 amino acid stalk region and one in the D2 domain at Thr 225. NKp46 lacks an activating cytoplasmic component but through a transmembrane Arg residue associates with CD3ζ and FcεRIγ which can transmit a cell activating signal via their multiple ITAM containing domains. A number of viral hemagglutinin, neuraminidase-hemagglutinin proteins which become surface expressed following viral infection have been proposed as ligands. In addition, Vimentin binding by NKp46 following mycobacteria infection of monocytes has been described [44]. NKp46 binding of specific heparan sulfate proteoglycans has also been proposed as a ligand and may be critical for tumor cell killing but its relevance to virally infected cell killing is unclear [45, 46].

The structure of NKp46 was determined by X-ray crystallography in 2003 [13, 14] revealing a structure made up of two C2-type Ig-like domains (Figure 4). Each domain is made up of eight β-strands which form two β-sheets with a typical disulphide bond formed between each sheet

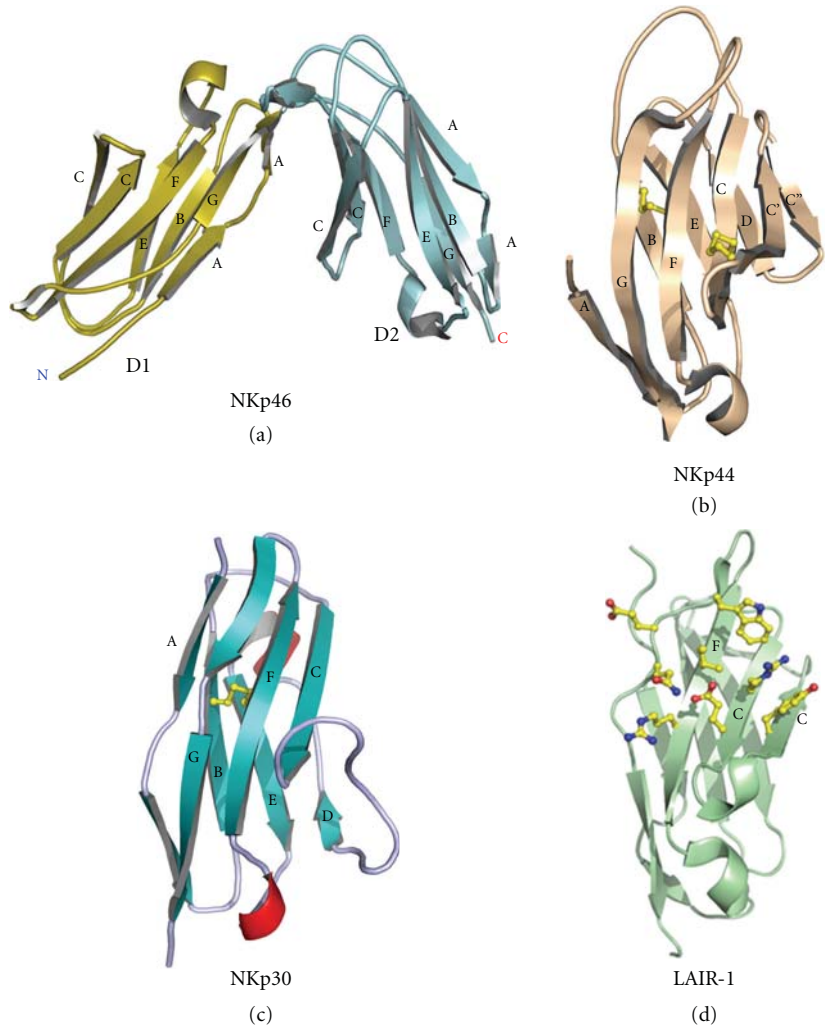

Figure 4: Structures of the natural cytotoxicity Receptors in cartoon representation; (a) NKp46, (b) NKp30 (c) NKp44. (d) Structure of human LAIR-1 with collagen binding residues shown in ball and stick representation.

maintaining the Ig-domain fold. The two domains are maintained at an angle of 85° respective to each other by a large number of hydrophobic interactions and extensive hydrogen bonding. An area of 1021 Å is buried between the two NKp46 domains illustrating the extensive interactions and within the core region of the domain interactions, 11 of 18 amino acids are conserved in NKp46 sequences from diverse species. Although associated by function with NKp30 and NKp44, NKp46 shows very low homology to the other natural cytotoxicity receptors (Figure 4). The most structurally similar molecules to NKp46 include the KIR family of proteins, ILT2 ([47] rmsd: 2.2 Å, hinge angle: 86°), glycoprotein VI ([48] rmsd: 1.6 Å, hinge angle: 90°), and also FcαRI ([49] CD89; rmsd: 2.4 Å; hinge angle: 92°). The majority of these homologous molecules have been structurally defined in complex with their respective ligands. All of these molecules have a very similar overall fold made up of two C2 type Ig-like domains and a hinge angle comparable to that observed in NKp46 (Figure 5). Altogether, the description of the binding sites of these homologous proteins suggests that NKp46 would also utilize the hinge region for ligand binding and the description of a ligand binding site as determined from structural studies or large-scale mutagenesis is eagerly anticipated.

NKp44 is a natural cytotoxicity receptor which is found on activated NK cells and leads to enhanced killing of both tumor cells and virally infected cells [50–52]. NKp44 is made up of a single extracellular IgV domain with a 64 amino acid stalk region which contains a number of glycosylation sites, a transmembrane region containing a lys residue, and a short cytoplasmic domain. Following NKp44 ligand binding, NK cell activation signals are transduced via DAP12 which associates with NKp44 through its transmembrane domain. The crystal structure of the extracellular domain of NKp44 displays a compact V domain structure [15]. It is made up of two typical β-sheets constructing the Ig-V domain structure. Unique features of this structure include the presence of a second disulphide bond which in combination with the atypical orientation of the equivalent CDR3 loop region creates a large grooved area on one face of the protein.

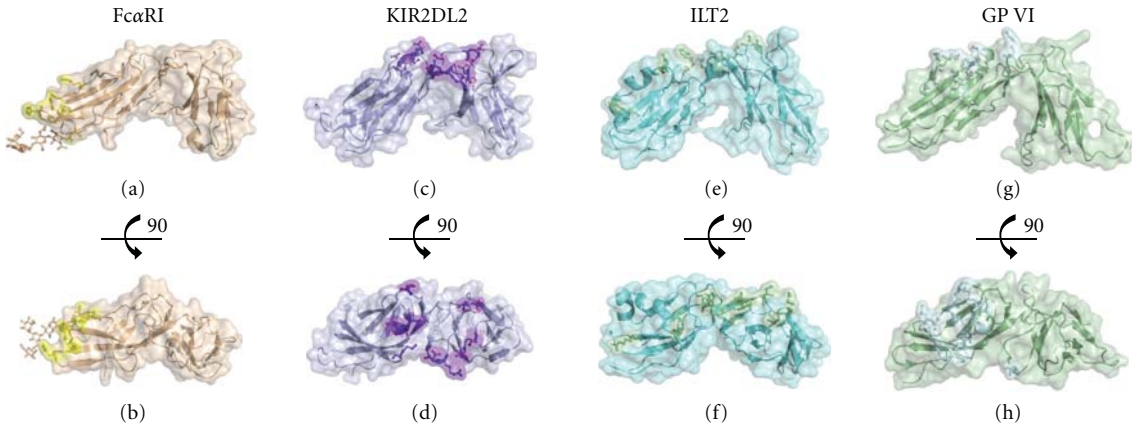

Figure 5: Structures of NKp46 homologous proteins ((a), (b)) FcαRI, ((c), (d)) KIR2DL2, ((e), (f)) ILT2, and ((g), (h)) GP VI shown in cartoon representation with transparent surface also shown. The known ligand binding residues of these molecules are given in ball and stick representation with the surface of these amino acids also highlighted. KIR binding to MHC molecules utilizes a number of residues found in the AB, CC′, EF loop regions of D1, the GA hinge region between D1 and D2, and the BC loop and FG loop regions of D2. ILT-2 uses residues located in the C strand, the CE loop region, EF loop region, and G strand of D1 while also using residues located in the BC loop region of D2. GP VI utilizes residues in the CC′ loop, C strand, CE loop, E strand, EF loop, and F strand of D1 while also using the FG loop region of D2. CD89 uses residues from the BC loop, C′ strand, C′E loop, and the FG loop of D1 in a side-on mechanism of binding in contrast to the other homologous proteins which all utilize the membrane distal face of the receptor for ligand binding. The lower panel shows the respective molecules in an "above-cell view" orientation which also highlights their ligand binding sites.

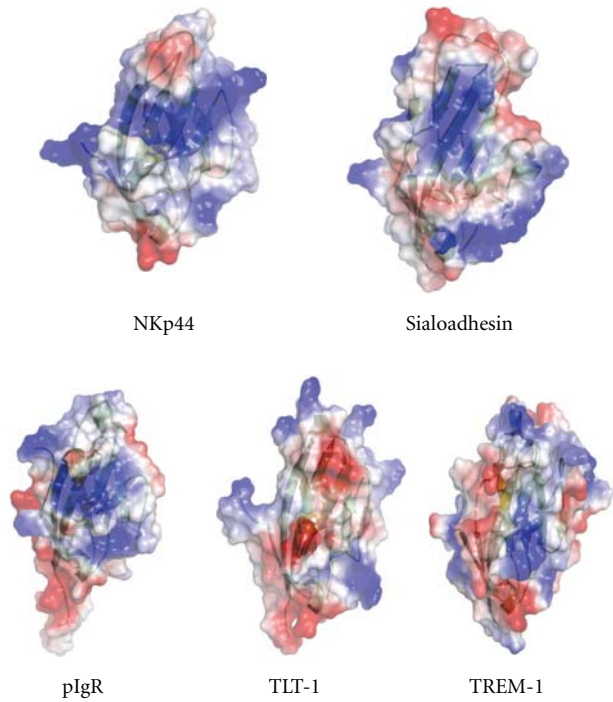

Figure 6: Charged surface representation of NKp44 and closely related homologues sialoadhesin, pIgR, TLT-1, and TREM-1. The orientation of each molecule is identical to that of NKp44 shown in Figure 4 and allows a view of the G, F, C, and C′ sheets of each molecule.

This groove is also positively charged and has been proposed as a ligand binding site. The NKp44 structure is homologous to a number of other Ig-like structures including TREM-1 (triggering receptor expressed on myeloid cells; rmsd: 2.0 Å) [53, 54], TREM-like transcript-1 (rmsd: 1.7 Å) [55], poly Ig receptor (rmsd: 2.1 Å) [56], sialoadhesin (rmsd: 2.4 Å) [57], and IREM-1 (inhibitory receptor expressed on myeloid cells, rmsd: 2.58 Å) [58] (Figure 6).

NKp44 binding to influenza hemagglutinin and other viral hemagglutinin-neuraminidase proteins due to the presence of sialic acid in the NKp44 stalk region have been presented in the literature [35, 50]. Since NKp44 is also

involved in the increased lysis of tumor cells, it will likely bind to a cellular ligand utilizing its Ig-like domain. In addition to transformed cells, NKp44 has also been shown to bind to the cell surface of mycobacteria and other bacteria which may contain a similar or homologous ligand to that found on human cells [59].

The most recently discovered NCR is NKp30 which was identified by Moretta and coworkers [60]. NKp30 has been shown to be the dominant activating receptor responsible for the lysis of a number of tumor cell types [60]. In addition, NKp30 has been shown to cause the activation and expansion of resting NK cells upon interaction with DCs and to cause the death of imDCs [61]. NKp30 has a single extracellular Ig-like domain with a short stalk region (~5 aa), and a transmembrane domain which associates with CD3ζ homodimers through a charged transmembrane interaction. The physiological ligands for NKp30 remain a controversial issue as a large number of NKp30 interacting molecules have been proposed. These include a human cytomegalovirus tegument protein pp65 [62], duffy-binding-like- (DBL-) 1α of *Plasmodium falciparum* erythrocyte membrane protein-1 (*Pf*EMP-1) [63], leukocyte antigen-B-associated transcript 3 (BAT3) [64, 65], and a group of heparan sulfate/heparin molecules [45]. Most recently, NKp30 was shown to recognize a B7 family homolog (B7-H6) as its ligand [66]. Unlike the other protein ligands proposed, B7-H6 is expressed on a number of tumor cell lines, such as K562 and Raji, as well as on primary cancer cells. However, the lack of structural information for NKp30 makes it difficult to parse out the most important ligand involved in cancer destruction from those proposed. The structure of NKp30 was recently, determined within our group and is shown to be a single I-type Ig-like domain [16]. The structure displays strong structural similarity to the CD28 family of receptors. Mutagenesis work carried out on NKp30 and binding studies on the B7H6 ligand indicates that binding is very similar to that observed for PD-1/PD-L1 interactions and involves residues found on the upper portion of the F and C strands.

2.5. The Structure of LAIR-1. The inhibitory receptor LAIR-1 is found on a large number of immune cells including NK cells and is specifically involved in collagen recognition [67] and increasing the threshold for NK cell activation. In contrast to LAIR-1, glycoprotein VI which is found on platelets also binds to collagen and through its association with the FcRγ chain, an activation signal is induced which leads to increased thrombosis. Recently the crystal structure of LAIR-1 was determined and the collagen binding site was mapped (Brondijk et al., 2010). LAIR-1 is made up of a single E-type Ig-like fold made up of two β-sheets consisting of β-strands ABE and A′GFCC′ with high structural homology to the D1 domain of KIR2DL2 (rmsd: 1.34 Å), ILT11 (rmsd: 1.36 Å), and GP VI (rmsd: 1.09 Å). Using mutagenesis and binding experiments in addition to NMR residue assignment signal shifts, it was possible to map the collagen binding site onto the A′FGCC′β-sheet specifically involving residues Arg59 and Glu61 from the LAIR-1 F strand and Trp109 and Glu111 from the G strand (Figure 4(d)). The collagen binding site of LAIR-1 is quite different to the site proposed for GP VI, and this may allow the design of molecules which can act as immune modulators by blocking LAIR-1 or affecting thrombosis via GP VI collagen binding.

3. C-Type Lectin-Like NK Receptors

Many NK receptor structures exhibit a C-type lectin fold, similar to that found among carbohydrate binding animal lectins and whose function requires bound calcium ions. Most C-type lectin-like NK receptors recognize membrane-bound protein ligands independent of carbohydrates and do not require calcium for their ligand recognition. Examples include CD69, CD94/NKG2 receptors which recognize HLA-E, NKG2D which recognizes stress-induced and tumor ligands such as MICA/B and ULBPs. In mouse, the classical class I MHC recognizing receptors, the Ly49 family receptors as well as Nkrp1 receptors, are also C-type lectin-like molecules. Within this review, we shall focus on the structures of human CD94/NKG2, NKG2D and their mechanisms of ligand recognition.

3.1. The Structure of the CD94/NKG2 Receptor and Its Recognition of HLA-E. CD94 exists primarily in a heterodimeric form with NKG2A, C, and E on the cell surface. Depending on the associated NKG2 subunit, CD94/NKG2 can function as either an activating (NKG2C, and E) or an inhibitory (NKG2A) receptor. The inhibitory form has intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIM), while the activating forms contain a positive transmembrane charged residue which facilitates interaction and signaling through the ITAM-containing DAP12 molecule. HLA-E was identified as the ligand for CD94/NKG2 [68–70]. Since C-type lectins are historically associated with carbohydrate binding, it was not clear how CD94/NKG2 would recognize HLA-E and whether the recognition involved the glycosylation of the class I MHC. In addition, the only class I binding receptors known at the time were T cell receptors, which are members of the immunoglobulin superfamily. Thus, part of the need to solve the CD94 structure was to understand how a C-type lectin fold receptor could recognize an MHC ligand. The crystal structure of a homodimeric human CD94 showed that the receptor maintained a canonical C-type lectin fold except that one of the two α-helices in the canonical C-type lectin fold (helix 2) was missing and replaced with a loop in the structure of CD94 (Figure 7(a)) [17]. More importantly, the CD94 structure showed that the receptor is missing four of the five calcium binding ligands and does not have a bound calcium ion in its putative calcium binding site. Thus, the structural identification suggested that the C-type lectin-like NK receptors functioned differently from a typical lectin calcium and carbohydrate binding molecule.

CD94/NKG2A binding of HLA-E by NK cells is critical for the careful monitoring of MHC class I expression on healthy cells. HLA-E molecules present peptides which have been generated from digested MHC class I molecules and the CD94/NKG2A complex detects this HLA-E/MHC class I peptide complex. This mechanism serves as a double-check to ensure that MHC class I molecules are being produced by a cell in a normal manner. Upon NKG2A/CD94 ligand

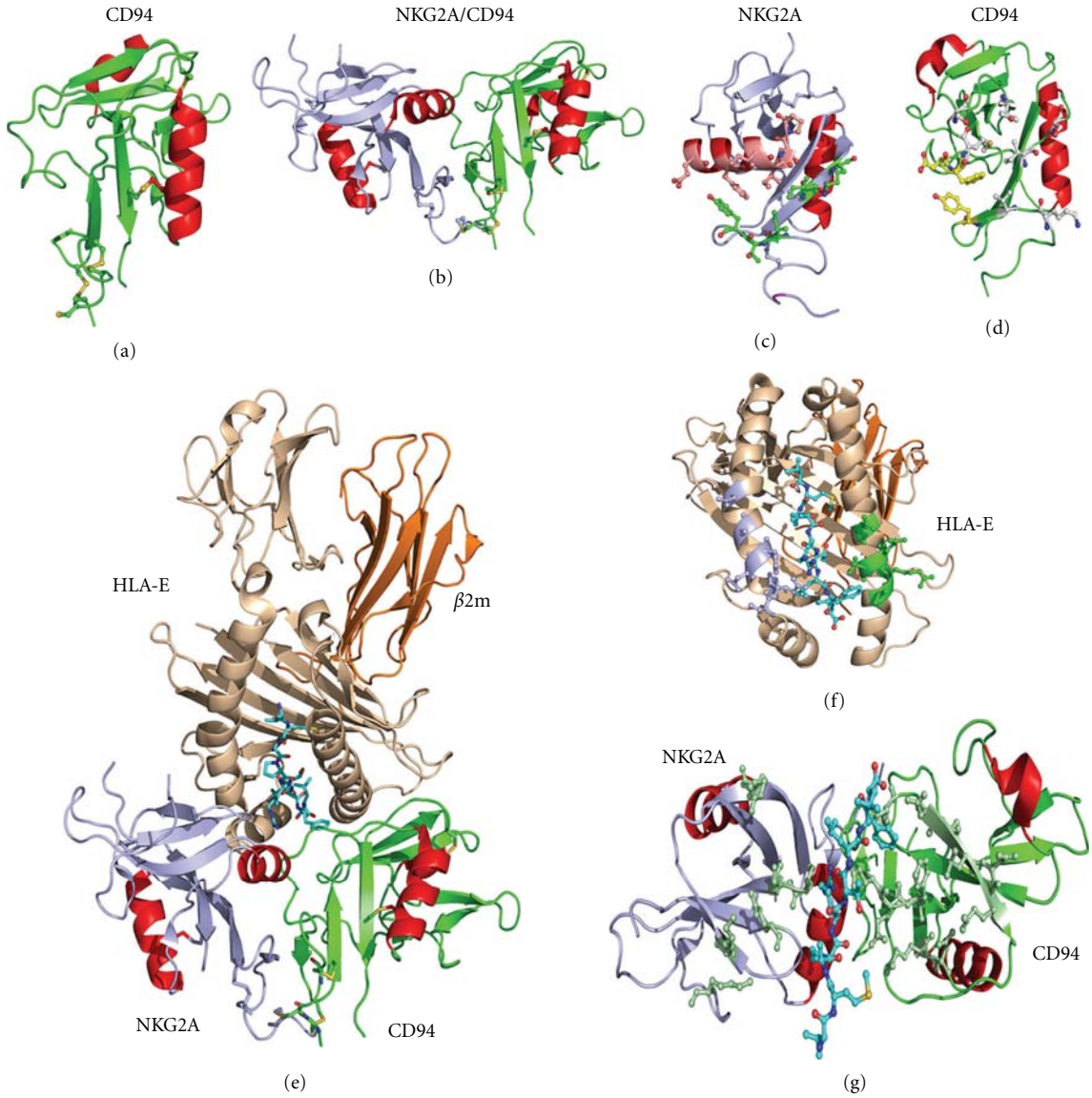

Figure 7: Structure of CD94 and associated complexes. (a) CD94 monomer shown in cartoon representation. (b) NKG2A/CD94 heterodimer structure with NKG2A shown in light blue and CD94 shown in green cartoon representation with each α-helix from both molecules colored red. (c) NKG2A shown in cartoon representation rotated 90° in relation to (7B) displaying interacting residues in ball and stick representation. Residues colored in green are part of interaction region 1 described in the text while residues colored salmon are part of region 2 also described in the text. (d) CD94 in cartoon representation rotated 90° in relation to (B) with residues colored yellow part of interaction region 1 while residues colored in grey are part of interaction region 2. (e) Cartoon representation of NKG2A/CD94 in complex with peptide bound HLA-E molecule. The bound peptide is shown in ball and stick representation with off-white carbon atoms (f) HLA-E molecule rotated 90° from (7E) illustrating the HLA-E interaction surface with residues which form bonds with NKG2A and CD94 shown in light blue coloring, and green coloring, respectively. Bound peptide is also shown in ball and stick representation. (g) NKG2A/CD94 molecule rotated 90° from (7E) showing the HLA-E interaction surface with the bound peptide shown for reference, residues which interact with HLA-E and the peptide are shown colored green in ball and stick representation.

binding of the HLA-E/peptide complex an inhibitory signal is transduced via cytoplasmic ITIM motifs found on NKG2A. The crystal structure of CD94/NKG2A first showed us how this heterodimeric complex is constructed [18]. The dimer interface between NKG2A and CD94 is extensive (1500 Å) and is made up of largely polar interactions with salt bridge formation observed at the edge of the dimer interface and a further central hydrophobic area maintaining the dimer (Figures 7(b), 7(c), and 7(d)). CD94/NKG2A dimerization is also driven by extensive main chain interactions between the β1 strands of both molecules, thus leading to the formation of a β-sheet made up of three β-strands from one molecule and three from the adjacent molecule.

Most recently, the structure of CD94/NKG2A in complex with HLA-E with a bound HLA-G peptide has been determined [19, 20]. The structure of CD94/NKG2A does

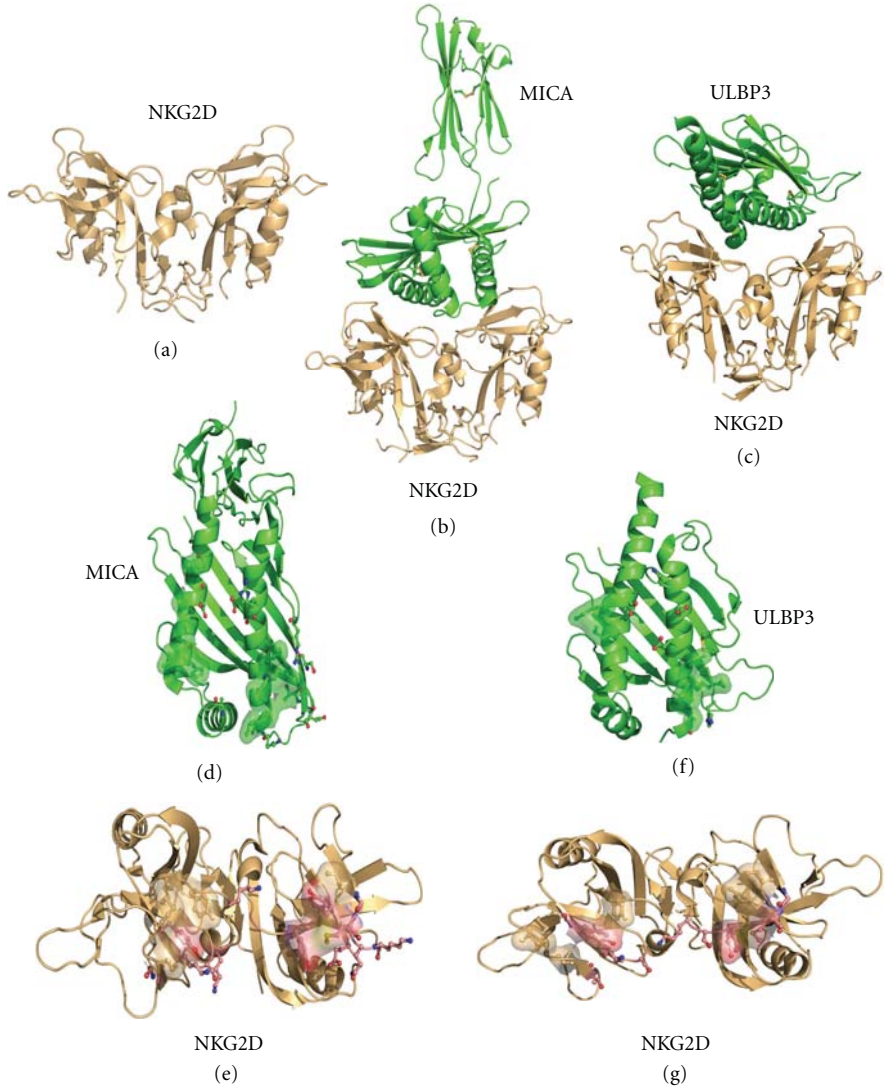

Figure 8: Structure of NKG2D homodimer and associated complexes. (a) NKG2D homodimeric structure. (b) NKG2D in the same orientation as (a) in complex with the two domain MICA molecule. (c) NKG2D in complex with ULBP3. (d) MICA structure rotated 90° in orientation relative to (b); residues forming hydrogen bonds with NKG2D are shown in ball and stick representation with red carbon and blue nitrogen atoms while residues forming hydrophobic interactions are shown in yellow transparent surface representation. (e) NKG2D structure rotated 90° in orientation relative to (b); residues forming hydrogen bonds with MICA are shown in ball and stick representation with red carbon and blue nitrogen atoms while residues forming hydrophobic interactions are shown in green transparent surface representation. (f) ULBP3 structure rotated 90° in orientation relative to (c); residues forming hydrogen bonds with NKG2D are shown in ball and stick representation with red carbon and blue nitrogen atoms while residues forming hydrophobic interactions are shown in yellow transparent surface representation. (g) NKG2D structure rotated 90° in orientation relative to (b); residues forming hydrogen bonds with ULBP3 are shown in ball and stick representation with red carbon and blue nitrogen atoms while residues forming hydrophobic interactions are shown in green transparent surface representation.

not undergo any structural rearrangements upon ligand binding, indicating that a lock and key mechanism of binding is used. NKG2A and CD94 interact with the $\alpha 2$ and $\alpha 1$ helices of HLA-E, respectively, with the presence of charge complementarity between the ligand and receptor clearly evident such that 8 salt bridges and 19 H bonds are observed; there is also a small hydrophobic patch in the ligand receptor interface. The buried surface area is very large (2100 Å) with the majority of it contributed by CD94. In addition, the surface complementarity is higher for CD94 interactions with HLA-E (0.68 versus 0.31 for NKG2A). Overall, the peptide makes up 23% of the ligand receptor interface, and again CD94 is dominant in its interactions with the peptide (80%) with CD94 interacting with the P5-Arg, P6-Thr, and P8-Phe while NKG2A only interacts with the P5-Arg.

3.2. Activating Receptor NKG2D and Its Ligand Bound Structures. The first NKG2D structure reported was that of the murine NKG2D homodimer [21], and, subsequently, both human and murine NKG2D structures were reported

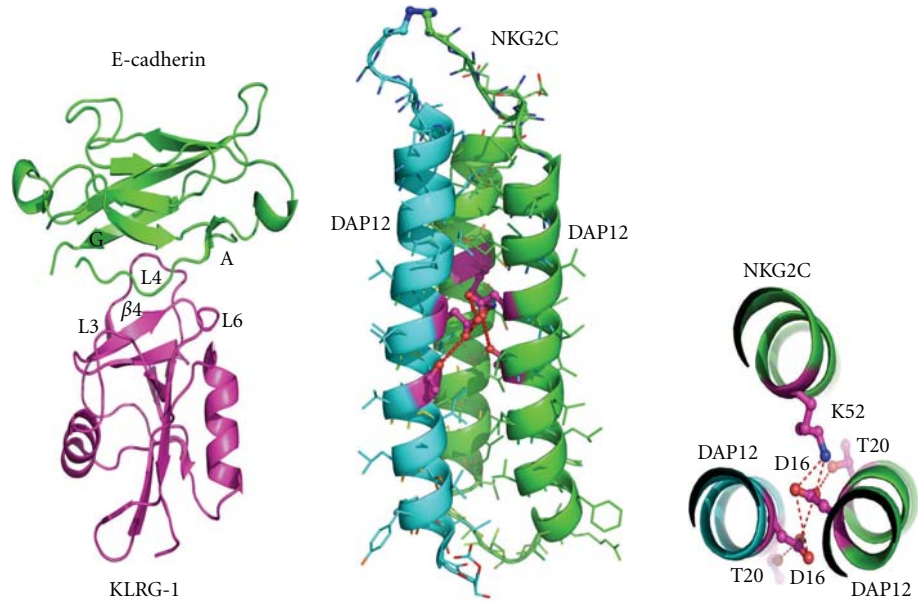

Figure 9: (a) Structure of KLRG1 in complex with E-cadherin shown in cartoon representation with the critical KLRG-1 loop regions which interact with the E-cadherin A′ and G sheet regions labeled. (b) Cartoon representation of the heterotrimeric NKG2C DAP12 transmembrane complex with interacting residues shown in ball and stick representation with bonding network shown by dashed lines. (c) View from above the membrane showing a closeup of the interacting residues and bonding interactions.

in complexes with their respective ligands, including ULBP3, MICA, and Rae-1β [22–24]. Similar to the CD94 receptor, the second α-helix in NKG2D is also distorted into a one-turn helix (Figure 8(a)) and the receptor does not have a functional calcium binding site, which was also demonstrated for the mouse C-type lectin-like Ly49 family of receptors [71].

NKG2D binds to a number of proteins which have high structural homology to the α1 and α2 domains of MHC class I molecules. Although the overall orientation of the NKG2D ligand bound structures are highly similar (Figure 8), there are very specific differences in the residues which interact with MICA compared to those seen in the ULBP3 complex. The homodimeric structure of NKG2D forms a central concave surface on its membrane distal face which allows the elevated central α-helices of the MHC class I homologues to bind NKG2D. The major binding region between the ligands MICA and ULBP3 and NKG2D receptor involves the c-terminal part of the α1 helix and the n-terminal region of the α3 helix of the ligands (Figure 8). NKG2D utilizes identical loop regions from both of its subunits to bind the ligand, and there is minimal structural reorganization following ligand binding. The binding interface between NKG2D ligands is extensive (1930 Å ULBP3 complex and 2180 Å MICA complex) and also quite specific (shape complementarity: 0.65 ULBP3 complex; 0.72 MICA complex) resulting in strong binding between the receptor and ligands. Analysis of the orientation of the two complexes indicates there is a subtle difference in the angle of orientation of ULBP3 and MICA on NKG2D resulting in a 6° difference. Interestingly, due to this slight change in the binding, it results in a difference in the number of hydrogen bonds between the complexes even though the strongest hydrogen bonds are conserved. In addition to the hydrogen bonding pattern, there is also an extensive hydrophobic region involved in ligand binding.

3.3. Other C-Type Lectin-Like NK Cell Receptors. In addition to the well-studied inhibitory molecules KIR proteins and also the collagen binding inhibitory molecule LAIR-1, killer cell lectin-like receptor G1 (KLRG1) is an additional inhibitory molecule [72]. KLRG1 contains an extracellular c-type lectin-like receptor, a 19 amino acid stalk region, a single transmembrane domain, and a cytoplasmic ITIM domain allowing the elicitation of an inhibitory signal within NK cells [73]. KLRG1 is found on the vast majority of NK cells, and, interestingly, surface expression is highly upregulated following viral or parasitic infection [74]. Most recently KLRG1 has been shown to interact with E-, N, and R-cadherins [75]. E-cadherin is a critical molecule found in the junction between epithelial cells where it mediates tight contacts while N- and R-cadherins perform similar functions with different cell types. In the presence of KLRG1/E-cadherin interactions, NK cell lysis is prevented. Relevant to NK surveillance of transformed cells, epithelial cells which have become malignant typically have reduced cadherin expression which in turn promotes tumor invasiveness and subsequent metastasis [76]. The crystal structure of KLRG1 in complex with E-cadherin has recently been determined and provides a clear understanding of KLRG interaction with E-cadherin [25]. KLRG-1 binds to E-cadherin with a relatively low affinity (100–200 μM). KLRG1 structure is

similar to other c-type lectin domains such as NKG2D made up of two alpha helices, two antiparallel beta sheets and the presence of intermolecular disulphide bonds.

KLRG1 binds to E-cadherin by using three loop regions (L3, L4, and L6) and β strand 4 which interact with the cadherin short A′ and G β strands similar to other CTLD receptors such as NKG2D with the binding characterized by exemplary surface complementarity and an extensive hydrogen bonding network (Figure 9(a)). The low affinity of KLRG-1 to a single cadherin domain may be overcome by multiple copies of KLRG-1 binding to the multiple domains of E-cadherin.

4. Transmembrane Signaling Complexes

Following ligand binding NK cell receptors initiate a signaling cascade which results in cell activation or inhibition. Critical to NK cell activation is the association of the transmembrane region of NK receptors with a signaling molecule containing an ITAM motif or association with adaptor molecules with a YXXM motif. The association of a NK activating receptor with an ITAM-containing adaptor molecule is mediated through transmembrane interactions and has recently been structurally characterized [26]. Utilizing solution NMR, the heterotrimeric structure of the transmembrane association of DAP12 with NKG2C was determined. The signaling assembly made up of a sophisticated electrostatic network involves a pair of aspartate residues from the DAP12 dimer, two adjacent threonine residues which together form the specific interaction with the lysine residue of NKG2C (Figures 9(b) and 9(c)). Mutagenesis and sequence analysis indicates that a very similar mechanism of heterotrimeric association occurs in the NKG2D-DAP10 complex formation. The interaction of CD16 with the CD3ζ and FcεRIγ as well as a number of other NK cell receptor and adaptor complexes is currently not structurally characterized, and this information will provide detailed understanding of the critical passage from ligand binding to cell activation.

This review has outlined the current state of human NK cell receptor structural biology. The structures which have been determined allow us to gain an understanding of the complex mechanisms involved in ligand recognition and NK cell activation and inhibition. Further high-resolution structure studies are likely over the next few years and remain eagerly anticipated in an effort to answer a number of burning questions within NK cell biology.

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References

[1] R. Biassoni, C. Bottino, C. Cantoni, and A. Moretta, "Human natural killer receptors and their ligands," in *Current Protocols in Immunology*, chapter 14: unit 14.10, 2002.

[2] L. L. Lanier, "NK cell recognition," *Annual Review of Immunology*, vol. 23, pp. 225–274, 2005.

[3] L. L. Lanier, "Up on the tightrope: natural killer cell activation and inhibition," *Nature Immunology*, vol. 9, no. 5, pp. 495–502, 2008.

[4] W. M. Yokoyama, "Natural killer cell immune responses," *Immunologic Research*, vol. 32, no. 1–3, pp. 317–325, 2005.

[5] Y. T. Bryceson, M. E. March, H. G. Ljunggren, and E. O. Long, "Activation, coactivation, and costimulation of resting human natural killer cells," *Immunological Reviews*, vol. 214, pp. 73–91, 2006.

[6] Q. R. Fan, L. Mosyak, C. C. Winter, N. Wagtmann, E. O. Long, and D. C. Wiley, "Structure of the inhibitory receptor for human natural killer cells resembles haematopoietic receptors," *Nature*, vol. 389, pp. 96–100, 1997.

[7] T. Graef, A. K. Moesta, P. J. Norman et al., "KIR2DS4 is a product of gene conversion with KIR3DL2 that introduced specificity for HLA-A*11 while diminishing avidity for HLA-C," *Journal of Experimental Medicine*, vol. 206, no. 11, pp. 2557–2572, 2009.

[8] K. Maenaka, T. Juji, D. I. Stuart, and E. Y. Jones, "Crystal structure of the human p58 killer cell inhibitory receptor (KIR2DL3) specific for HLA-Cw3-related MHC class I," *Structure*, vol. 7, no. 4, pp. 391–398, 1999.

[9] X. Saulquin, L. N. Gastinel, and E. Vivier, "Crystal structure of the human natural killer cell activating receptor KIR2DS2 (CD158j)," *Journal of Experimental Medicine*, vol. 197, no. 7, pp. 933–938, 2003.

[10] G. A. Snyder, A. G. Brooks, and P. D. Sun, "Crystal structure of the HLA-Cw3 allotype-specific killer cell inhibitory receptor KIR2DL2," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 7, pp. 3864–3869, 1999.

[11] J. C. Boylngton, S. A. Motykat, P. Schuck, A. G. Brooks, and P. D. Sun, "Crystal structure of an NK cell immunoglobulin-like receptor in complex with its class I MHC ligand," *Nature*, vol. 405, no. 6786, pp. 537–543, 2000.

[12] Q. R. Fan, E. O. Long, and D. C. Wiley, "Crystal structure of the human natural killer cell inhibitory receptor KIR2DL1-HLA-Cw4 complex," *Nature Immunology*, vol. 2, no. 5, pp. 452–460, 2001.

[13] C. E. Foster, M. Colonna, and P. D. Sun, "Crystal structure of the human natural killer (NK) cell activating receptor NKp46 reveals structural relationship to other leukocyte receptor complex immunoreceptors," *The Journal of Biological Chemistry*, vol. 278, no. 46, pp. 46081–46086, 2003.

[14] M. Ponassi, C. Cantoni, R. Biassoni et al., "Structure of the human NK cell triggering receptor NKp46 ectodomain," *Biochemical and Biophysical Research Communications*, vol. 309, no. 2, pp. 317–323, 2003.

[15] C. Cantoni, M. Ponassi, R. Biassoni et al., "The three-dimensional structure of the human NK cell receptor NKp44, a triggering partner in natural cytotoxicity," *Structure*, vol. 11, no. 6, pp. 725–734, 2003.

[16] M. G. Joyce, P. Tran, M. A. Zhuravleva, J. Jaw, and P. D. Sun, "Crystal structure of human natural cytotoxicity receptor NKp30 and identification of its ligand binding site," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 15, pp. 6223–6228, 2011.

[17] J. C. Boyington, A. N. Riaz, A. Patamawenu, J. E. Coligan, A. G. Brooks, and P. D. Sun, "Structure of CD94 reveals novel C-type lectin fold: implications for the NK cell-associated CD94/NKG2 receptors," *Immunity*, vol. 10, no. 1, pp. 75–82, 1999.

[18] L. C. Sullivan, C. S. Clements, T. Beddoe et al., "The heterodimeric assembly of the CD94-NKG2 receptor family and implications for human leukocyte antigen-E recognition," *Immunity*, vol. 27, no. 6, pp. 900–911, 2007.

[19] E. J. Petrie, C. S. Clements, J. Lin et al., "CD94-NKG2A recognition of human leukocyte antigen (HLA)-E bound to an HLA class I leader sequence," *Journal of Experimental Medicine*, vol. 205, no. 3, pp. 725–735, 2008.

[20] B. K. Kaiser, J. C. Pizarro, J. Kerns, and R. K. Strong, "Structural basis for NKG2A/CD94 recognition of HLA-E," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 18, pp. 6696–6701, 2008.

[21] D. W. Wolan, L. Teyton, M. G. Rudolph et al., "Crystal structure of the murine NK cell-activating receptor NKG2D at 1.95 A," *Nature Immunology*, vol. 2, no. 3, pp. 248–254, 2001.

[22] P. Li, D. L. Morris, B. E. Willcox, A. Steinle, T. Spies, and R. K. Strong, "Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA," *Nature Immunology*, vol. 2, no. 5, pp. 443–451, 2001.

[23] S. Radaev, B. Rostro, A. G. Brooks, M. Colonna, and P. D. Sun, "Conformational plasticity revealed by the cocrystal structure of NKG2D and its class i MHC-like ligand ULBP3," *Immunity*, vol. 15, no. 6, pp. 1039–1049, 2001.

[24] P. Li, G. McDermott, and R. K. Strong, "Crystal structures of RAE-1beta and its complex with the activating immunoreceptor NKG2D," *Immunity*, vol. 16, no. 1, pp. 77–86, 2002.

[25] Y. Li, M. Hofmann, Q. Wang et al., "Structure of natural killer cell receptor KLRG1 bound to E-cadherin reveals basis for MHC-independent missing self recognition," *Immunity*, vol. 31, no. 1, pp. 35–46, 2009.

[26] M. E. Call, K. W. Wucherpfennig, and J. J. Chou, "The structural basis for intramembrane assembly of an activating immunoreceptor complex," *Nature Immunology*, vol. 11, pp. 1023–1029, 2010.

[27] C. C. Winter and E. O. Long, "A single amino acid in the p58 killer cell inhibitory receptor controls the ability of natural killer cells to discriminate between the two groups of HLA-C allotypes," *Journal of Immunology*, vol. 158, no. 9, pp. 4026–4028, 1997.

[28] O. Mandelboim, H. T. Reyburn, M. Vales-Gomez et al., "Protection from lysis by natural killer cells of group 1 and 2 specificity is mediated by residue 80 in human histocompatibility leukocyte antigen C alleles and also occurs with empty major histocompatibility complex molecules," *Journal of Experimental Medicine*, vol. 184, no. 3, pp. 913–922, 1996.

[29] S. I. Khakoo, R. Rajalingam, B. P. Shum et al., "Rapid evolution of NK cell receptor systems demonstrated by comparison of chimpanzees and humans," *Immunity*, vol. 12, no. 6, pp. 687–698, 2000.

[30] D. Middleton, L. Menchaca, H. Rood, and R. Komerofsky, "New allele frequency database: www.allelefrequencies.net," *Tissue Antigens*, vol. 61, no. 5, pp. 403–407, 2003.

[31] M. Stern, H. Elsasser, G. Honger, J. Steiger, S. Schaub, and C. Hess, "The number of activating KIR genes inversely correlates with the rate of CMV infection/reactivation in kidney transplant recipients," *American Journal of Transplantation*, vol. 8, no. 6, pp. 1312–1317, 2008.

[32] S. E. Hiby, R. Apps, A. M. Sharkey et al., "Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2," *Journal of Clinical Investigation*, vol. 120, no. 11, pp. 4102–4110, 2010.

[33] A. Moretta, C. Bottino, M. Vitale et al., "Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis," *Annual Review of Immunology*, vol. 19, pp. 197–223, 2001.

[34] S. Sivori, M. Vitale, L. Morelli et al., "p46, a novel natural killer cell-specific surface molecule that mediates cell activation," *Journal of Experimental Medicine*, vol. 186, no. 7, pp. 1129–1136, 1997.

[35] M. Jarahian, C. Watzl, P. Fournier et al., "Activation of natural killer cells by newcastle disease virus hemagglutinin-neuraminidase," *Journal of Virology*, vol. 83, no. 16, pp. 8108–8121, 2009.

[36] E. Cagnano, O. Hershkovitz, A. Zilka et al., "Expression of ligands to NKp46 in benign and malignant melanocytes," *Journal of Investigative Dermatology*, vol. 128, no. 4, pp. 972–979, 2008.

[37] V. Yutkin, D. Pode, E. Pikarsky, and O. Mandelboim, "The expression level of ligands for natural killer cell receptors predicts response to bacillus Calmette-Guerin therapy: a pilot study," *Journal of Urology*, vol. 178, no. 6, pp. 2660–2664, 2007.

[38] R. Castriconi, A. Dondero, F. Negri et al., "Both CD133+ and CD133- medulloblastoma cell lines express ligands for triggering NK receptors and are susceptible to NK-mediated cytotoxicity," *European Journal of Immunology*, vol. 37, pp. 3190–3196, 2007.

[39] Y. M. El-Sherbiny, J. L. Meade, T. D. Holmes et al., "The requirement for DNAM-1, NKG2D, and NKp46 in the natural killer cell-mediated killing of myeloma cells," *Cancer Research*, vol. 67, pp. 8444–8449, 2007.

[40] C. L. Fuller, G. Ruthel, K. L. Warfield et al., "NKp30-dependent cytolysis of filovirus-infected human dendritic cells," *Cellular Microbiology*, vol. 9, no. 4, pp. 962–976, 2007.

[41] S. E. Chisholm, K. Howard, M. V. Gómez, and H. T. Reyburn, "Expression of ICP0 is sufficient to trigger natural killer cell recognition of herpes simplex virus-infected cells by natural cytotoxicity receptors," *Journal of Infectious Diseases*, vol. 195, no. 8, pp. 1160–1168, 2007.

[42] S. E. Chisholm and H. T. Reyburn, "Recognition of vaccinia virus-infected cells by human natural killer cells depends on natural cytotoxicity receptors," *Journal of Virology*, vol. 80, no. 5, pp. 2225–2233, 2006.

[43] O. Mandelboim, N. Lieberman, M. Lev et al., "Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells," *Nature*, vol. 409, no. 6823, pp. 1055–1060, 2001.

[44] A. Garg, P. F. Barnes, A. Porgador et al., "Vimentin expressed on Mycobacterium tuberculosis-infected human monocytes is involved in binding to the NKp46 receptor," *Journal of Immunology*, vol. 177, no. 9, pp. 6192–6198, 2006.

[45] M. L. Hecht, B. Rosental, T. Horlacher et al., "Natural cytotoxicity receptors NKp30, NKp44 and NKp46 bind to different heparan sulfate/heparin sequences," *Journal of Proteome Research*, vol. 8, no. 2, pp. 712–720, 2009.

[46] A. Zilka, G. Landau, O. Hershkovitz et al., "Characterization of the heparin/heparan sulfate binding site of the natural cytotoxicity receptor NKp46," *Biochemistry*, vol. 44, no. 44, pp. 14477–14485, 2005.

[47] B. E. Willcox, L. M. Thomas, and P. J. Bjorkman, "Crystal structure of HLA-A2 bound to LIR-1, a host and viral major histocompatibility complex receptor," *Nature Immunology*, vol. 4, no. 9, pp. 913–919, 2003.

[48] K. Horii, M. L. Kahn, and A. B. Herr, "Structural basis for platelet collagen responses by the immune-type receptor glycoprotein VI," *Blood*, vol. 108, no. 3, pp. 936–942, 2006.

[49] A. B. Herr, E. R. Ballister, and P. J. Bjorkman, "Insights into IgA-mediated immune responses from the crystal structure of human Fc-alpha-RI and its complex with IgA1-Fc," *Nature*, vol. 423, no. 6940, pp. 614–620, 2003.

[50] T. I. Arnon, M. Lev, G. Katz, Y. Chernobrov, A. Porgador, and O. Mandelboim, "Recognition of viral hemagglutinins by NKp44 but not by NKp30," *European Journal of Immunology*, vol. 31, no. 9, pp. 2680–2689, 2001.

[51] C. Cantoni, C. Bottino, M. Vitale et al., "NKp44, a triggering receptor involved in tumor cell lysis by activated human natural killer cells, is a novel member of the immunoglobulin superfamily," *Journal of Experimental Medicine*, vol. 189, no. 5, pp. 787–795, 1999.

[52] M. Vitale, C. Bottino, S. Sivori et al., "NKp44, a novel triggering surface molecule specifically expressed by activated natural killer cells, is involved in non-major histocompatibility complex-restricted tumor cell lysis," *Journal of Experimental Medicine*, vol. 187, no. 12, pp. 2065–2072, 1998.

[53] M. S. Kelker, E. W. Debler, and I. A. Wilson, "Crystal structure of mouse triggering receptor expressed on myeloid cells 1 (TREM-1) at 1.76 A," *Journal of Molecular Biology*, vol. 344, no. 5, pp. 1175–1181, 2004.

[54] S. Radaev, M. Kattah, B. Rostro, M. Colonna, and P. D. Sun, "Crystal structure of the human myeloid cell activating receptor TREM-1," *Structure*, vol. 11, no. 12, pp. 1527–1535, 2003.

[55] J. L. Gattis, A. V. Washington, M. M. Chisholm et al., "The structure of the extracellular domain of triggering receptor expressed on myeloid cells like transcript-1 and evidence for a naturally occurring soluble fragment," *Journal of Biological Chemistry*, vol. 281, no. 19, pp. 13396–13403, 2006.

[56] A. E. Hamburger, A. P. West Jr., and P. J. Bjorkman, "Crystal structure of a polymeric immunoglobulin binding fragment of the human polymeric immunoglobulin receptor," *Structure*, vol. 12, no. 11, pp. 1925–1935, 2004.

[57] N. R. Zaccai, A. P. May, R. C. Robinson et al., "Crystallographic and in silico analysis of the sialoside-binding characteristics of the Siglec sialoadhesin," *Journal of Molecular Biology*, vol. 365, no. 5, pp. 1469–1479, 2007.

[58] J. A. Marquez, E. Galfre, F. Dupeux, D. Flot, O. Moran, and N. Dimasi, "The crystal structure of the extracellular domain of the inhibitor receptor expressed on myeloid cells IREM-1," *Journal of Molecular Biology*, vol. 367, no. 2, pp. 310–318, 2007.

[59] S. Esin, G. Batoni, C. Counoupas et al., "Direct binding of human NK cell natural cytotoxicity receptor NKp44 to the surfaces of mycobacteria and other bacteria," *Infection and Immunity*, vol. 76, no. 4, pp. 1719–1727, 2008.

[60] D. Pende, S. Parolini, A. Pessino et al., "Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells," *Journal of Experimental Medicine*, vol. 190, no. 10, pp. 1505–1516, 1999.

[61] G. Ferlazzo, M. L. Tsang, L. Moretta, G. Melioli, R. M. Steinman, and C. Münz, "Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells," *Journal of Experimental Medicine*, vol. 195, no. 3, pp. 343–351, 2002.

[62] T. I. Arnon, H. Achdout, O. Levi et al., "Inhibition of the NKp30 activating receptor by pp65 of human cytomegalovirus," *Nature Immunology*, vol. 6, no. 5, pp. 515–523, 2005.

[63] E. Mavoungou, J. Held, L. Mewono, and P. G. Kremsner, "A duffy binding-like domain is involved in the NKp30-mediated recognition of Plasmodium falciparum-parasitized erythrocytes by natural killer cells," *Journal of Infectious Diseases*, vol. 195, no. 10, pp. 1521–1531, 2007.

[64] E. Pogge von Strandmann, V. R. Simhadri, B. von Tresckow et al., "Human leukocyte antigen-B-associated transcript 3 is released from tumor cells and engages the NKp30 receptor on natural killer cells," *Immunity*, vol. 27, no. 6, pp. 965–974, 2007.

[65] V. R. Simhadri, K. S. Reiners, H. P. Hansen et al., "Dendritic cells release HLA-B-associated transcript-3 positive exosomes to regulate natural killer function," *PLoS ONE*, vol. 3, no. 10, article e3377, 2008.

[66] C. S. Brandt, M. Baratin, E. C. Yi et al., "The B7 family member B7-H6 is a tumor cell ligand for the activating natural killer cell receptor NKp30 in humans," *Journal of Experimental Medicine*, vol. 206, no. 7, pp. 1495–1503, 2009.

[67] R. J. Lebbink, T. De Ruiter, J. Adelmeijer et al., "Collagens are functional, high affinity ligands for the inhibitory immune receptor LAIR-1," *Journal of Experimental Medicine*, vol. 203, no. 6, pp. 1419–1425, 2006.

[68] F. Borrego, M. Ulbrecht, E. H. Weiss, J. E. Coligan, and A. G. Brooks, "Recognition of human histocompatibility leukocyte antigen (HLA)-E complexed with HLA class I signal sequence-derived peptides by CD94/NKG2 confers protection from natural killer cell-mediated lysis," *Journal of Experimental Medicine*, vol. 187, no. 5, pp. 813–818, 1998.

[69] V. M. Braud, D. S. Allan, C. A. O'Callaghan et al., "HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C," *Nature*, vol. 391, no. 6669, pp. 795–799, 1998.

[70] N. Lee, M. Llano, M. Carretero et al., "HLA-E is a major ligand for the natural killer inhibitory receptor CD94/NKG2A," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 9, pp. 5199–5204, 1998.

[71] J. Tormo, K. Natarajan, D. H. Margulles, and R. A. Mariuzza, "Crystal structure of a lectin-like natural killer cell receptor bound to its MHC class I ligand," *Nature*, vol. 402, no. 6762, pp. 623–631, 1999.

[72] Y. T. Bryceson and E. O. Long, "Line of attack: NK cell specificity and integration of signals," *Current Opinion in Immunology*, vol. 20, no. 3, pp. 344–352, 2008.

[73] M. D. Guthmann, M. Tal, and I. Pecht, "A secretion inhibitory signal transduction molecule on mast cells is another C-type lectin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 20, pp. 9397–9401, 1995.

[74] D. Voehringer, M. Kaufmann, and H. Pircher, "Genomic structure, alternative splicing, and physical mapping of the killer cell lectin-like receptor G1 gene (KLRG1), the mouse homologue of MAFA," *Immunogenetics*, vol. 52, no. 3-4, pp. 206–211, 2001.

[75] M. Ito, T. Maruyama, N. Saito, S. Koganei, K. Yamamoto, and N. Matsumoto, "Killer cell lectin-like receptor G1 binds three members of the classical cadherin family to inhibit NK cell cytotoxicity," *Journal of Experimental Medicine*, vol. 203, no. 2, pp. 289–295, 2006.

[76] M. Colonna, "Cytolytic responses: cadherins put out the fire," *Journal of Experimental Medicine*, vol. 203, no. 2, pp. 261–264, 2006.

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Review Article

Interactions of Human Myeloid Cells with Natural Killer Cell Subsets In Vitro and In Vivo

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In both human and mouse it has been recently realized that natural killer (NK) cells do not emerge from the bone marrow with full functional competence but rather acquire functions in interaction with antigen-presenting cells (APCs), primarily dendritic cells (DCs). Here we review the mechanisms and the consequences of this NK-cell preactivation, as well as discuss new experimental models that now allow investigating these interactions for human NK cells and their response to human pathogens in vivo. These investigations will allow harnessing NK cells during vaccination for improved innate and adaptive immunity.

1. Introduction

Myeloid cells and natural killer (NK) cells constitute two types of innate leucocytes that restrict infections early on and then influence adaptive immune responses to pathogen invasion, so that B and T cells efficiently clear invading microorganisms. In recent years it has become apparent that myeloid and NK cells perform their tasks not in isolation but influence and activate each other for more efficient innate immunity [1]. Particularly, dendritic cells (DCs), which have long been realized to be essential sentinels to detect infections for priming of adaptive T-cell responses [2], have recently been shown to be crucial for efficient NK-cell responses [3]. The diversity of DC subsets with migratory, secondary lymphoid tissue resident, and inflammatory DCs developing from monocytes [4] allows for a fine-tuned activation of NK cells at different tissue sites. In addition, maturation of these DC subsets upon encounter of different pathogen-associated molecular patterns (PAMPs) equips them with NK-cell stimulatory abilities adjusting to the need of stronger or weaker NK-cell activation in the course of an immune response [5]. These mature DCs migrate or are resident in secondary lymphoid tissues (Figure 1), a major site of NK-cell activation during innate immune responses [3, 6]. While in mice NK cells seem to need to home there during immune responses [3, 7], human secondary lymphoid tissues contain substantial amounts of NK cells [8, 9]. Thus, NK-cell activation in secondary lymphoid tissues by mature DCs allows for the activation of innate lymphocytes, which limit pathogen replication prior to priming of adaptive immune responses by the same antigen-presenting cells (APCs) at the same sites.

Once activated, NK cells contribute to the mounting immune response primarily via cytokine production and cytotoxicity. In humans, these two functions can be mediated by different NK-cell differentiation stages with $CD56^{bright}CD16^{-}$ NK cells preferentially producing cytokines upon activation by APCs and terminally differentiated $CD56^{dim}CD16^{+}$ NK cells being potent killers of virus- infected and-transformed cells [10, 11]. Interestingly, $CD56^{bright}CD16^{-}$ NK cells, which primarily react to activation by mature DCs with cytokine production, with elevation of the cytotoxic ability and with expansion, are enriched in T-cell zones of secondary lymphoid organs [8, 9]. Thus, human NK-cell distribution seems to be adjusted to ensure efficient activation by DCs to rapidly mobilize the NK-cell compartment during the initial phase of immune responses to infections.

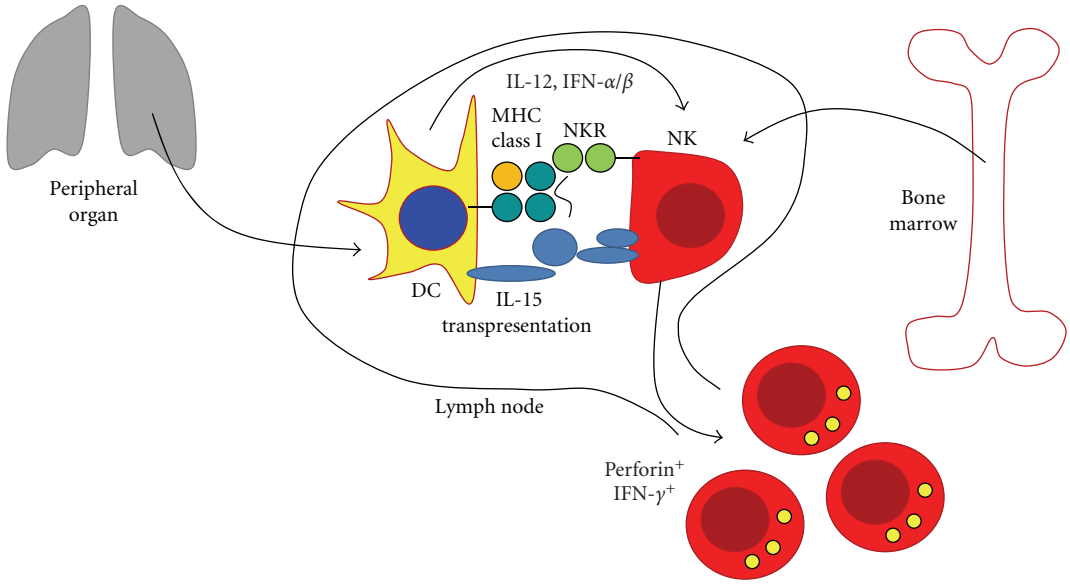

Figure 1: NK-cell preactivation in secondary lymphoid organs by dendritic cells (DCs). Mature DCs preactivate NK cells in secondary lymphoid tissues but are not killed in the process and continue to prime adaptive immune responses. They achieve this by simultaneously transmitting IL-15-dependent activating signals via transpresentation of this cytokine in distinct domains of their synapse with DCs, while inhibiting NK-cell lysis by engaging inhibitory receptors via MHC class I molecules occurs in other domains of the synapse center. In addition, DC-derived IL-12 and type I IFN (IFN-α/β) increase IFN-γ production and cytotoxicity of conjugated NK cells. NK cells home to these sites after development in the bone marrow, while mature DCs migrate there at increased frequency from peripheral tissues after their maturation.

The present paper will summarize recent studies providing information about the molecular interactions that take place between NK cells and myeloid antigen-presenting cells and how these interactions can lead to completely different outcomes for the ensuing immune responses, ranging from immune suppression after APC editing to enhanced Th1-polarized immune responses.

2. NK-Cell Activation by DCs—Synapses and Cytokines

However, this rapid mobilization of cytotoxic innate effector cells bears the risk of compromising efficient priming of adaptive immune response due to APC killing. Indeed, NK cells in C57BL/6 mice, which carry an activating NK-cell receptor, Ly49H, directly engaging the viral m157 protein of mouse cytomegalovirus (MCMV), kill MCMV-infected DCs so efficiently that ensuing adaptive immune responses are compromised and have difficulties controlling persistent viral infection [12]. However, in most other instances moderate NK-cell responses can be activated by mature DCs, which then go on to prime protective adaptive immune responses. This is achieved by protecting mature DCs from NK-cell cytotoxicity during NK-cell activation, and the immunological synapse, which mediates these interactions, fulfills both functions. Indeed, two mechanisms have been described by which mature DCs protect themselves from NK-cell cytotoxicity. On the one hand, mature DCs express serpin protease inhibitors, like protease inhibitor 9 (PI9), that inhibit apoptosis inducing proteases, like granzyme B, which are delivered to targets of NK-cell cytotoxicity via perforin pores [13, 14]. On the other hand, DC maturation upregulates surface expression of major histocompatibility complex (MHC) class I molecules, which serve as ligands of inhibitory receptors on NK cells of the CD94/NKG2 and KIR families (Figure 1). Indeed, blocking of MHC class I molecules restores mature DC killing by NK cells to levels observed with immature DCs [15]. Furthermore, mature DCs rapidly polarize these MHC class I molecules to their synapses with NK cells to ensure inhibition of cytotoxicity [16]. Therefore, mature DCs ensure in their interaction with NK cells that they survive to allow successive priming of adaptive immune responses.

Parallel to these inhibitory interactions, mature DCs, however, activate resting NK cells [15, 17–19], and this interaction is crucial for mounting efficient NK-cell responses [3]. DC cytokine secretion or transpresented cytokines play a major role in this NK-cell activation. While IL-12 and IL-18 primarily stimulate cytokine production, for example of IFN-γ, by NK cells [5, 6], type I IFN is mainly involved in the augmentation of NK-cell cytotoxicity [20]. In addition, IL-15, presented by IL-15Rα on mature DCs [6], can stimulate NK-cell survival, proliferation, and differentiation from $CD56^{bright}CD16^{-}$ to $CD56^{dim}CD16^{+}$ NK cells [6, 8, 16, 21, 22]. Indeed, IL-15 is crucial for DC-mediated activation of NK cells in mice [3]. Interestingly, these activating interactions, especially IL-15 transpresentation to IL-15Rβ/γ_c, between mature DCs and resting NK cells seem to be mediated through distinct domains in the center of

the immunological synapse (Figure 1) [16]. These activating domains segregate from inhibitory domains that allow MHC class I/KIR interactions and form rapidly within the first minutes of mature DC interaction with NK cells. The rapid establishment of the immunological synapse between mature DCs and resting NK cells, which ensures NK-cell activation and DC protection from NK-cell cytotoxicity at the same time, seems to be important, because in vivo imaging has revealed that DCs and NK cells might only interact with each other in T-cell areas of secondary lymphoid tissues for short periods of time with the majority of contacts being around 1min [23, 24]. At these early time points, however, the synapse between DCs and NK cells is not stabilized by cytoskeletal components like actin. This occurs similarly to T cells only after 20 min and might be required to exchange all necessary signals between these two innate leucocyte populations. Especially, IL-12 might only efficiently polarize to the synapse at these late time points, and efficient cytokine production by NK cells might therefore require longer interactions with DCs [25]. This late NK-cell activation might be further modulated by IL-18 released from DCs [26]. Thus, human NK cells, especially those resident in secondary lymphoid tissues, might entertain longer interactions with DCs to reach their full functional potential and might even differentiate into $CD56^{dim}CD16^{+}$ NK cells upon this interaction.

3. Myeloid Cell Editing by NK Cells—Cytotoxicity and Activation

While we consider NK cells primarily as effector cells, activated early after sensing of infections by DCs, it is worthwhile to consider the flip side of their interaction with DCs, namely, DC maturation by activated NK cells as well as NK-cell regulation of other myeloid cells. In vitro activated human NK cells can induce phenotypic maturation of DCs via their secretion of IFN-γ and TNF-α, and these changes are cell-contact dependent [18, 19, 27]. Monocytes have been shown to secrete more TNF-α in the presence of activated NK cells in a process that also was shown to require cell-to-cell contact [28], and polymorphonuclear cells increase their phagocytic activity and are more resistant to apoptosis when conditioned with supernatant from cytokine-activated NK cells [29]. Even the differentiation of $CD14^{+}$ monocytes into DCs with typical functional attributes was reported to be induced by NK cells and was mediated by GM-CSF production and CD154 expression by the $CD56^{bright}$ NK-cell subset [30]. This shaping of the dendritic cell fate extends to the killing of immature DCs by activated autologous NK cells in certain in vitro settings with involvement of CD40-CD40L interactions and the activating NK-cell receptors (NCRs) NKp30, NKp46, and DNAM-1, whereas increased expression of MHC class I molecules on matured DCs protects them from NK-cell-mediated cytotoxicity [15, 19, 31–35]. Degranulation of NK cells, a surrogate for cytotoxic activity, can be observed against human cytomegalovirus-infected DCs, with dominant contributions of NKp46 and DNAM-1 and exerted mainly by $CD94/NKG2A^{+}$ NK cells [36], as also reported for the cytotoxicity towards immature DCs [34]. Human dendritic cells infected with influenza virus increase cytotoxicity of NK cells towards autologous immature DCs but are themselves spared from NK-cell-mediated cytolysis, possibly due to upregulation of MHC class I molecules [37]. As for mature DCs, upregulation of MHC class I molecules after treatment with LPS protects human microglial cells—resident macrophages of the central nervous system—from NK-cell cytotoxicity [38]. Nonactivated microglial cells on the other hand are efficiently killed by activated NK cells mediated in part by the activating NCRs NKp46 and DNAM-1 [38]. Conversely to DCs and microglia, high-dose LPS-activated human macrophages are prone to cytolysis mediated by autologous NK cells compared to less activated macrophages [39], and this is also influenced by the polarization status of macrophages [40] as well as the pathway of macrophage activation [41]. This NK-cell cytotoxicity against activated macrophages was reported to be partly dependent on IL-10-induced upregulation of NKG2D ligands [42]. Thus, NK cells can edit myeloid antigen-presenting cells via different mechanisms, and different myeloid cells are differently affected. While low numbers of activated NK cells mature DCs via cytokine secretion, high numbers kill immature DCs, resting microglia, and activated macrophages.

These in vitro defined interactions probably form the basis for the modulation of adaptive immune responses in vivo. Indeed, in the draining lymph nodes of mice, recruitment of NK cells and NK-cell-secreted IFN-γ seem to be essential for the establishment of Th1 responses [7]. This NK-cell-assisted Th1 polarization renders immune responses more efficient in the clearance of *Leishmania* infection [43, 44]. Furthermore, depletion of NK cells leads to altered phenotype and numbers of DCs in lymph nodes [45, 46], and this can lead to changes in the capacity to prime T-cell responses [12]. Interestingly, in the early phase of mouse cytomegalovirus infection—a mouse model for persistent viral infections—, infected DCs are killed by activated NK cells, and this culling of antigen-presenting cells diminished both antiviral $CD8^{+}$ and $CD4^{+}$ T-cell responses which led to an inability to clear the virus in the long run [12]. Likewise, NK-cell-mediated elimination of DCs in lymph nodes of transplantation models inhibited alloreactive T-cell priming [46, 47]. Furthermore, targeting of microglia by NK cells reduced disease severity in experimental autoimmune encephalomyelitis models [48, 49] via suppression of detrimental Th17 responses [49]. While, therefore, abundant activated NK cells can edit APCs to inhibit immune responses, lower amounts of activated NK cells can mature APCs and thereby use NK-cell recognition of cells, altered by transformation or infection, to initiate adaptive immune responses even upstream of professional APCs like DCs. Along these lines, NK cells, activated in vivo by tumor cell lines with low levels of inhibitory MHC class I molecules, initiated DC-mediated priming of protective T cells and adaptive memory generation [50]. In a similar setting, antigen-expressing NK-cell targets induced specific cellular as well as humoral adaptive immune responses, possibly involving increased uptake and antigen presentation by myeloid APCs like DCs [51]. Thus, NK cells can influence adaptive immune

Table 1: Distribution and frequencies of NK cells, NK-cell subsets, and myeloid cells in mice with reconstituted human immune system components. Only data from two mouse model systems of human immune system reconstitution are shown. Frequency of NK-cell subsets relates to total NK cells. In vivo administration of human cytokines is reported to lead to up to tenfold increases in targeted cell compartments [22, 52]. *Chijioke et al., unpublished.

	huNSG	huRag2$^{-/-}\gamma_c^{-/-}$
NK cells (CD3$^-$NKp46$^+$ or CD3$^-$CD56$^+$)	*Bone marrow*: 0.5–1.5% [52, 54] *Blood*: 1–3% [52, 54] *Spleen*: 1–3% [52, 54] *Thymus*: not reported *Lung*: 2–5% [52, 54] *Liver*: 3–4.5% [52, 54]	*Bone marrow:* <0.2% [22] *Blood:* <0.5% [22] *Spleen:* <0.2% [22] *Thymus:* <1% [22] *Lung*: not reported *Liver:* <0.4% [22]
CD56^{bright}CD16$^-$ NK cells	*Bone marrow*: >30% [*] *Blood*: 25% [*] *Spleen*: 5–10% [54, *] *Thymus*: not reported *Liver*: 5% [*]	*Bone marrow*: 10% [22] *Blood*: not reported *Spleen*: 40% [22] *Thymus*: 30% [22] *Liver*: not reported
CD56^{dim}CD16$^+$ NK cells	*Bone marrow*: <15% [*] *Blood*: 20% [*] *Spleen*: 30–40% [54, *] *Thymus*: not reported *Liver*: 30% [*]	*Bone marrow*: 90% [22] *Blood*: not reported *Spleen*: 60% [22] *Thymus*: 70% [22] *Liver*: not reported
CD56$^-$ NK cells	*Bone marrow*: <10% [*] *Blood*: 30% [*] *Spleen*: 30–40% [54, *] *Thymus*: not reported *Liver*: 40–50% [*]	*Bone marrow*: % not reported [22] *Blood*: not reported *Spleen*: % not reported [22] *Thymus*: % not reported [22] *Liver*: not reported
CD123$^+$HLA-DR$^+$ plasmacytoid DCs	*Bone marrow*: not reported *Spleen*: 1–2% [54]	*Bone marrow*: 1.5–3% [22, 55] *Spleen*: <0.5% [55]
CD11c$^+$HLA-DR$^+$ myeloid DCs	*Bone marrow*: 1–3% [56] *Blood*: not reported *Spleen*: 1–3% [54, 56]	*Bone marrow*: 1–3% [22, 55] *Blood*: 5% [57] *Spleen*: <2% [55]
CD141$^+$HLR-DR$^+$ myeloid DCs	*Spleen*: <0,1% [58]	Not reported
CD33$^+$ myeloid cells	*Bone marrow*: 5–15% [56] *Blood*: 5–10% [56] *Spleen*: 5–10% [56]	*Bone marrow*: 20% [57] *Blood*: not reported *Spleen*: not reported
CD14$^+$ monocytes/macrophages	*Bone marrow*: 6–10% [52, 59] *Blood*: 2–5% [52, 60] *Spleen*: 1–3% [52, 59] *Lung*: 10–20% [52] *Liver*: 5–10% [52]	*Bone marrow*: 2% [57] *Blood*: 15% [57] *Spleen*: not reported *Lung*: not reported *Liver*: not reported
CD66$^+$ granulocytes	Not reported	*Bone marrow*: 10% [57] *Blood*: 5% [57]
CD15$^+$ neutrophils	*Bone marrow*: 4% [59] *Spleen*: 1% [59]	Not reported

*Chijioke et al., unpublished.

responses by at least three mechanisms. They can provide a favorable cytokine milieu for Th1 priming by DCs, they can kill subsets of APCs to edit or even inhibit immune responses, and, finally, they have the capacity to even initiate immune activation after stimulation by somatic cells.

4. NK-Cell Preactivation and Distribution In Vivo

The in vitro studies described in the previous paragraph examining the interplay between human NK cells and myeloid cells mostly utilized activated NK cells, mainly activated with cytokines (in most cases IL-2), while nonactivated or resting NK cells had lesser or no regulatory function on the interacting myeloid cells. Equally, in mouse antiviral immune responses in vivo, only activated NK cells, by virtue of binding of the activating NK-cell receptor Ly49H with the virus-encoded protein m157 on infected cells, were able to alter antiviral T-cell responses through the killing of DCs but not NK cells lacking the Ly49H receptor [12]. Studies trying to assess the in vivo function of human NK cells using mice with reconstituted human immune system components found that these demonstrated dampened effector functions,

however, increased to full functional competence of the NK-cell compartment after preactivation with IL-15 or poly(I:C) ex vivo [22, 52–54]. IL-15 was able to directly preactivate NK cells, while poly(I:C) required splenic bystander cells to do so, suggesting that TLR3, although expressed by human NK cells, is not able to fully activate human NK cells in vivo [54]. Preactivation of human NK cells also enhanced clearing of transferred MHC class I-deficient tumor cells in vivo [52, 54]. In addition to preactivation, IL-15, and especially complexes of IL-15 with IL-15Rα, were able to induce terminal differentiation of human NK cells and significantly expanded these innate lymphocytes in vivo by promoting the development of $CD56^{dim}CD16^{+}$ cells from their $CD56^{bright}CD16^{-}$ precursors [22, 52]. Thus, similar to mice [3], human NK cells require preactivation by IL-15 to reach their functional capacity in vivo. However, it is likely that this cytokine is not produced in sufficient amounts by the human hematopoietic cell lineages reconstituting in these in vivo models and therefore hinders reconstitution of similar frequencies of terminally differentiated NK cells as seen in humans.

With respect to the production of IL-15, human myeloid reconstitution and the location of human IL-15 producing cells might be crucial. In this respect, terminal differentiation of human NK cells was suggested to take place in secondary lymphoid organs [61], and it should be important how well these tissues are reconstituted with human myeloid cells, especially macrophages and DCs. An overview of human myeloid and probably myeloid cell-dependent NK-cell reconstitution in different organs of mice with reconstituted human immune system components is given in Table 1. While human myeloid cell reconstitution can be observed in peripheral blood and spleen, reaching nearly similar frequencies of human DCs and around one-third of the frequency of human monocytes/macrophages compared to the splenic and peripheral blood populations of these cells in humans, secondary lymphoid organogenesis is compromised in these mice, because of the deficiency in γ_c-chain expression [57, 58, 60]. Often only the mesenteric lymph node can be macroscopically observed in these mice. Thus, enhanced myeloid reconstitution and measures to promote secondary lymphoid tissue development in these mice could also increase total NK-cell numbers as well as their terminal differentiation. Additional modifications of mice with reconstituted human immune system components along these lines could allow the more faithful modeling of innate immune responses in these in vivo systems of the human immune system.

5. Conclusions

Innate lymphocyte activation by DCs has now become an integral function of these APCs in addition to the originally recognized priming of adaptive immunity by these cells [1]. Especially NK cells, which are efficient innate effector cells against tumors and virus-infected cells, are attractive targets of this activation and desirable to be harnessed during vaccination. As discussed above, such recruitment of NK-cell effector functions during immunization would both directly target infected and transformed cells, as well as influence the adaptive immune response to vaccine antigens and to antigenic spreading after NK-cell-mediated killing.

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References

[1] C. Münz, R. M. Steinman, and S. I. Fujii, "Dendritic cell maturation by innate lymphocytes: coordinated stimulation of innate and adaptive immunity," *Journal of Experimental Medicine*, vol. 202, no. 2, pp. 203–207, 2005.

[2] R. M. Steinman, "Dendritic cells in vivo: a key target for a new vaccine science," *Immunity*, vol. 29, no. 3, pp. 319–324, 2008.

[3] M. Lucas, W. Schachterle, K. Oberle, P. Aichele, and A. Diefenbach, "Dendritic cells prime natural killer cells by trans-presenting interleukin 15," *Immunity*, vol. 26, no. 4, pp. 503–517, 2007.

[4] W. R. Heath and F. R. Carbone, "Dendritic cell subsets in primary and secondary T cell responses at body surfaces," *Nature Immunology*, vol. 10, no. 12, pp. 1237–1244, 2009.

[5] T. Strowig, F. Brilot, F. Arrey et al., "Tonsilar NK cells restrict B cell transformation by the epstein-barr virus via IFN-γ," *PLoS Pathogens*, vol. 4, no. 2, article e27, 2008.

[6] G. Ferlazzo, M. Pack, D. Thomas et al., "Distinct roles of IL-12 and IL-15 in human natural killer cell activation by dendritic cells from secondary lymphoid organs," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 47, pp. 16606–16611, 2004.

[7] A. Martín-Fontecha, L. L. Thomsen, S. Brett et al., "Induced recruitment of NK cells to lymph nodes provides IFN-γ for T1 priming," *Nature Immunology*, vol. 5, no. 12, pp. 1260–1265, 2004.

[8] G. Ferlazzo, D. Thomas, S. L. Lin et al., "The abundant NK cells in human secondary lymphoid tissues require activation to express killer cell Ig-like receptors and become cytolytic," *Journal of Immunology*, vol. 172, no. 3, pp. 1455–1462, 2004.

[9] T. A. Fehniger, M. A. Cooper, G. J. Nuovo et al., "$CD56^{bright}$ natural killer cells are present in human lymph nodes and are activated by T cell-derived IL-2: a potential new link between adaptive and innate immunity," *Blood*, vol. 101, no. 8, pp. 3052–3057, 2003.

[10] G. Ferlazzo and C. Münz, "Dendritic cell interactions with NK cells from different tissues," *Journal of Clinical Immunology*, vol. 29, no. 3, pp. 265–273, 2009.

[11] A. Lünemann, J. D. Lünemann, and C. Münz, "Regulatory NK-cell functions in inflammation and autoimmunity," *Molecular Medicine*, vol. 15, no. 9-10, pp. 352–358, 2009.

[12] D. M. Andrews, M. J. Estcourt, C. E. Andoniou et al., "Innate immunity defines the capacity of antiviral T cells to limit persistent infection," *Journal of Experimental Medicine*, vol. 207, no. 6, pp. 1333–1343, 2010.

[13] C. E. Hirst, M. S. Buzza, C. H. Bird et al., "The intracellular granzyme B inhibitor, proteinase inhibitor 9, is up-regulated

during accessory cell maturation and effector cell degranulation, and its overexpression enhances CTL potency," *Journal of Immunology*, vol. 170, no. 2, pp. 805–815, 2003.

[14] J. P. Medema, D. H. Schuurhuis, D. Rea et al., "Expression of the serpin serine protease inhibitor 6 protects dendritic cells from cytotoxic T lymphocyte-induced apoptosis: differential modulation by T helper type 1 and type 2 cells," *Journal of Experimental Medicine*, vol. 194, no. 5, pp. 657–667, 2001.

[15] G. Ferlazzo, M. L. Tsang, L. Moretta, G. Melioli, R. M. Steinman, and C. Münz, "Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells," *Journal of Experimental Medicine*, vol. 195, no. 3, pp. 343–351, 2002.

[16] F. Brilot, T. Strowig, S. M. Roberts, F. Arrey, and C. Münz, "NK cell survival mediated through the regulatory synapse with human DCs requires IL-15Rα," *Journal of Clinical Investigation*, vol. 117, no. 11, pp. 3316–3329, 2007.

[17] N. C. Fernandez, A. Lozier, C. Flament et al., "Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses in vivo," *Nature Medicine*, vol. 5, no. 4, pp. 405–411, 1999.

[18] F. Gerosa, B. Baldani-Guerra, C. Nisii, V. Marchesini, G. Carra, and G. Trinchieri, "Reciprocal activating interaction between natural killer cells and dendritic cells," *Journal of Experimental Medicine*, vol. 195, no. 3, pp. 327–333, 2002.

[19] D. Piccioli, S. Sbrana, E. Melandri, and N. M. Valiante, "Contact-dependent stimulation and inhibition of dendritic cells by natural killer cells," *Journal of Experimental Medicine*, vol. 195, no. 3, pp. 335–341, 2002.

[20] F. Gerosa, A. Gobbi, P. Zorzi et al., "The reciprocal interaction of NK cells with plasmacytoid or myeloid dendritic cells profoundly affects innate resistance functions," *Journal of Immunology*, vol. 174, no. 2, pp. 727–734, 2005.

[21] C. Münz, T. Dao, G. Ferlazzo, M. A. De Cos, K. Goodman, and J. W. Young, "Mature myeloid dendritic cell subsets have distinct roles for activation and viability of circulating human natural killer cells," *Blood*, vol. 105, no. 1, pp. 266–273, 2005.

[22] N. D. Huntington, N. Legrand, N. L. Alves et al., "IL-15 trans-presentation promotes human NK cell development and differentiation in vivo," *Journal of Experimental Medicine*, vol. 206, no. 1, pp. 25–34, 2009.

[23] K. R. Garrod, S. H. Wei, I. Parker, and M. D. Cahalan, "Natural killer cells actively patrol peripheral lymph nodes forming stable conjugates to eliminate MHC-mismatched targets," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 29, pp. 12081–12086, 2007.

[24] H. Beuneu, J. Deguine, B. Breart, O. Mandelboim, J. P. Di Santo, and P. Bousso, "Dynamic behavior of NK cells during activation in lymph nodes," *Blood*, vol. 114, no. 15, pp. 3227–3234, 2009.

[25] C. Borg, A. Jalil, D. Laderach et al., "NK cell activation by dendritic cells (DCs) requires the formation of a synapse leading to IL-12 polarization in DCs," *Blood*, vol. 104, no. 10, pp. 3267–3275, 2004.

[26] C. Semino, G. Angelini, A. Poggi, and A. Rubartelli, "NK/iDC interaction results in IL-18 secretion by DCs at the synaptic cleft followed by NK cell activation and release of the DC maturation factor HMGB1," *Blood*, vol. 106, no. 2, pp. 609–616, 2005.

[27] M. Vitale, M. Della Chiesa, S. Carlomagno et al., "NK-dependent DC maturation is mediated by TNFα and IFNγ released upon engagement of the NKp30 triggering receptor," *Blood*, vol. 106, no. 2, pp. 566–571, 2005.

[28] N. Dalbeth, R. Gundle, R. J. O. Davies, Y. C. G. Lee, A. J. McMichael, and M. F. C. Callan, "CD56^{bright} NK cells are enriched at inflammatory sites and can engage with monocytes in a reciprocal program of activation," *Journal of Immunology*, vol. 173, no. 10, pp. 6418–6426, 2004.

[29] N. Bhatnagar, H. S. Hong, J. K. Krishnaswamy et al., "Cytokine-activated NK cells inhibit PMN apoptosis and preserve their functional capacity," *Blood*, vol. 116, no. 8, pp. 1308–1316, 2010.

[30] A. L. Zhang, P. Colmenero, U. Purath et al., "Natural killer cells trigger differentiation of monocytes into dendritic cells," *Blood*, vol. 110, no. 7, pp. 2484–2493, 2007.

[31] J. L. Wilson, L. C. Heffler, J. Charo, A. Scheynius, M. T. Bejarano, and H. G. Ljunggren, "Targeting of human dendritic cells by autologous NK cells," *Journal of Immunology*, vol. 163, no. 12, pp. 6365–6370, 1999.

[32] E. Carbone, G. Terrazzano, G. Ruggiero et al., "Recognition of autologous dendritic cells by human NK cells," *European Journal of Immunology*, vol. 29, no. 12, pp. 4022–4029, 1999.

[33] G. M. Spaggiari, R. Carosio, D. Pende et al., "NK cell-mediated lysis of autologous antigen-presenting cells is triggered by the engagement of the phosphatidylinositol 3-kinase upon ligation of the natural cytotoxicity receptors NKp30 and NKp46," *European Journal of Immunology*, vol. 31, no. 6, pp. 1656–1665, 2001.

[34] M. D. Chiesa, M. Vitale, S. Carlomagno, G. Ferlazzo, L. Moretta, and A. Moretta, "The natural killer cell-mediated killing of autologous dendritic cells is confined to a cell subset expressing CD94/NKG2A, but lacking inhibitory killer Ig-like receptors," *European Journal of Immunology*, vol. 33, no. 6, pp. 1657–1666, 2003.

[35] D. Pende, R. Castriconi, P. Romagnani et al., "Expression of the DNAM-1 ligands, Nectin-2 (CD112) and poliovirus receptor (CD155), on dendritic cells: relevance for natural killer-dendritic cell interaction," *Blood*, vol. 107, no. 5, pp. 2030–2036, 2006.

[36] G. Magri, A. Muntasell, N. Romo et al., "NKp46 and DNAM-1 NK-cell receptors drive the response to human cytomegalovirus-infected myeloid dendritic cells overcoming viral immune evasion strategies ," *Blood*, vol. 117, no. 3, pp. 848–856, 2011.

[37] M. Draghi, A. Pashine, B. Sanjanwala et al., "NKp46 and NKG2D recognition of infected dendritic cells is necessary for NK cell activation in the human response to influenza infection," *Journal of Immunology*, vol. 178, no. 5, pp. 2688–2698, 2007.

[38] A. Lünemann, J. D. Lünemann, S. Roberts et al., "Human NK cells kill resting but not activated microglia via NKG2D- and NKp46-mediated recognition," *Journal of Immunology*, vol. 181, no. 9, pp. 6170–6177, 2008.

[39] S. Nedvetzki, S. Sowinski, R. A. Eagle et al., "Reciprocal regulation of human natural killer cells and macrophages associated with distinct immune synapses," *Blood*, vol. 109, no. 9, pp. 3776–3785, 2007.

[40] F. Bellora, R. Castriconi, A. Dondero et al., "The interaction of human natural killer cells with either unpolarized or polarized macrophages results in different functional outcomes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 50, pp. 21659–21664, 2010.

[41] P. Eissmann, J. H. Evans, M. Mehrabi, E. L. Rose, S. Nedvetzki, and D. M. Davis, "Multiple mechanisms downstream of TLR-4 stimulation allow expression of NKG2D ligands to facilitate macrophage/NK cell crosstalk," *Journal of Immunology*, vol. 184, no. 12, pp. 6901–6909, 2010.

[42] U. Schulz, M. Kreutz, G. Multhoff et al., "Interleukin-10 promotes NK cell killing of autologous macrophages by stimulating expression of NKG2D ligands," *Scandinavian Journal of Immunology*, vol. 72, no. 4, pp. 319–331, 2010.

[43] M. Bajénoff, B. Breart, A. Y. C. Huang et al., "Natural killer cell behavior in lymph nodes revealed by static and real-time imaging," *Journal of Experimental Medicine*, vol. 203, no. 3, pp. 619–631, 2006.

[44] Y. Laouar, F. S. Sutterwala, L. Gorelik, and R. A. Flavell, "Transforming growth factor-β controls T helper type 1 cell development through regulation of natural killer cell interferon-γ," *Nature Immunology*, vol. 6, no. 6, pp. 600–607, 2005.

[45] R. Winkler-Pickett, H. A. Young, J. M. Cherry et al., "In vivo regulation of experimental autoimmune encephalomyelitis by NK cells: alteration of primary adaptive responses," *Journal of Immunology*, vol. 180, no. 7, pp. 4495–4506, 2008.

[46] S. Laffont, C. Seillet, J. Ortaldo, J. D. Coudert, and J.-C. Guéry, "Natural killer cells recruited into lymph nodes inhibit alloreactive T-cell activation through perforin-mediated killing of donor allogeneic dendritic cells," *Blood*, vol. 112, no. 3, pp. 661–671, 2008.

[47] G. Yu, X. Xu, D. V. Minh, E. D. Kilpatrick, and C. L. Xian, "NK cells promote transplant tolerance by killing donor antigen-presenting cells," *Journal of Experimental Medicine*, vol. 203, no. 8, pp. 1851–1858, 2006.

[48] J. W. Leavenworth, C. Schellack, H. J. Kim, L. Lu, P. Spee, and H. Cantor, "Analysis of the cellular mechanism underlying inhibition of EAE after treatment with anti-NKG2A F(ab′)," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 6, pp. 2562–2567, 2010.

[49] J. Hao, R. Liu, W. Piao et al., "Central nervous system (CNS)-resident natural killer cells suppress Th17 responses and CNS autoimmune pathology," *Journal of Experimental Medicine*, vol. 207, no. 9, pp. 1907–1921, 2010.

[50] R. Mocikat, H. Braumüller, A. Gumy et al., "Natural killer cells activated by MHC class I^{low} targets prime dendritic cells to induce protective CD8 T cell responses," *Immunity*, vol. 19, no. 4, pp. 561–569, 2003.

[51] P. Krebs, M. J. Barnes, K. Lampe et al., "NK cell-mediated killing of target cells triggers robust antigen-specific T cell-mediated and humoral responses," *Blood*, vol. 113, no. 26, pp. 6593–6602, 2009.

[52] Q. Chen, M. Khoury, and J. Chen, "Expression of human cytokines dramatically improves reconstitution of specific human-blood lineage cells in humanized mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 51, pp. 21783–21788, 2009.

[53] M. C. André, A. Erbacher, C. Gille et al., "Long-term human $CD34^+$ stem cell-engrafted nonobese diabetic/SCID/IL-$2R\gamma^{null}$ mice show impaired $CD8^+$ T cell maintenance and a functional arrest of immature NK cells," *Journal of Immunology*, vol. 185, no. 5, pp. 2710–2720, 2010.

[54] T. Strowig, O. Chijioke, P. Carrega et al., "Human NK cells of mice with reconstituted human immune system components require preactivation to acquire functional competence," *Blood*, vol. 116, no. 20, pp. 4158–4167, 2010.

[55] E. Traggiai, L. Chicha, L. Mazzucchelli et al., "Development of a human adaptive immune system in cord blood cell-transplanted mice," *Science*, vol. 304, no. 5667, pp. 104–107, 2004.

[56] F. Ishikawa, M. Yasukawa, B. Lyons et al., "Development of functional human blood and immune systems in NOD/SCID/IL2 receptor γ $chain^{null}$ mice," *Blood*, vol. 106, no. 5, pp. 1565–1573, 2005.

[57] J. Song, T. Willinger, A. Rongvaux et al., "A mouse model for the human pathogen salmonella typhi," *Cell Host and Microbe*, vol. 8, no. 4, pp. 369–376, 2010.

[58] L. F. Poulin, M. Salio, E. Griessinger et al., "Characterization of human $DNGR\text{-}1^+$ $BDCA3^+$ leukocytes as putative equivalents of mouse $CD8\alpha^+$ dendritic cells," *Journal of Experimental Medicine*, vol. 207, no. 6, pp. 1261–1271, 2010.

[59] J. Unsinger, J. S. McDonough, L. D. Shultz, T. A. Ferguson, and R. S. Hotchkiss, "Sepsis-induced human lymphocyte apoptosis and cytokine production in "humanized" mice," *Journal of Leukocyte Biology*, vol. 86, no. 2, pp. 219–227, 2009.

[60] T. Strowig, C. Gurer, A. Ploss et al., "Priming of protective T cell responses against virus-induced tumors in mice with human immune system components," *Journal of Experimental Medicine*, vol. 206, no. 6, pp. 1423–1434, 2009.

[61] C. Romagnani, K. Juelke, M. Falco et al., "$CD56^{bright}CD16^-$ killer Ig-like receptor NK cells display longer telomeres and acquire features of $CD56^{dim}$ NK cells upon activation," *Journal of Immunology*, vol. 178, no. 8, pp. 4947–4955, 2007.

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Review Article

Role of Natural Killer and Dendritic Cell Crosstalk in Immunomodulation by Commensal Bacteria Probiotics

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A cooperative dialogue between natural killer (NK) cells and dendritic cells (DCs) has been elucidated in the last years. They help each other to acquire their complete functions, both in the periphery and in the secondary lymphoid organs. Thus, NK cells' activation by dendritic cells allows the killing of transformed or infected cells in the periphery but may also be important for the generation of adaptive immunity. Indeed, it has been shown that NK cells may play a key role in polarizing a Th1 response upon interaction with DCs exposed to microbial products. This regulatory role of DC/NK cross-talk is of particular importance at mucosal surfaces such as the intestine, where the immune system exists in intimate association with commensal bacteria such as lactic acid bacteria (LAB). We here review NK/DC interactions in the presence of gut-derived commensal bacteria and their role in bacterial strain-dependent immunomodulatory effects. We particularly aim to highlight the ability of distinct species of commensal bacterial probiotics to differently affect the outcome of DC/NK cross-talk and consequently to differently influence the polarization of the adaptive immune response.

1. Introduction

Dendritic cells (DCs) and natural killer (NK) cells play a critical role in early defenses against cancer and infections, and evidence of interactions between these two cell types has accumulated in the last years [1–7]. This interaction might results in NK cell activation, DC maturation, or DC death, depending on the activation status of both cell types. Thus, the outcome of NK/DC crosstalk is likely to influence the innate as well as the subsequent adaptive immune responses [8]. This crosstalk can be promoted by pathogen-derived products that activate different innate immune cell types directly and simultaneously through their Toll-like receptors (TLRs) [9]. Indeed, DCs and NK cells have developed different, but partially overlapping, systems to identify pathogen-associated danger signals and they are, therefore, differently involved in the detection of various microorganisms.

DCs are critical for initiating immune responses against both pathogenic and nonpathogenic bacteria. In an immature stage, DCs reside in peripheral tissues, continuously sampling the microenvironment, sensing the presence of pathogens, and releasing chemokines and cytokines to amplify the immune response [10]. It has been clearly evidenced that, depending on the nature of the stimuli received, myeloid DCs can develop into different subsets that possess unique biological functions, determined by the combination of surface molecule expression and cytokine secretion [10]. In part, these different outcomes are influenced by exposure of the DCs to microbial products. Therefore, the regulatory role of DCs is of particular importance at mucosal surfaces such as the intestine, where the immune system exists in intimate association with the commensal bacteria such as lactic acid bacteria (LAB) [11]. Interestingly, recent studies have demonstrated that different strains of LAB posses the ability to finely regulate myeloid DCs maturation, polarizing

the subsequent T cell activity toward Th1, Th2, or even Treg responses [12–14].

Natural killer (NK) cells distinguish between normal healthy cells and abnormal cells by using a sophisticated repertoire of cell surface receptors [15, 16], playing a key role in the immune response to certain infections and malignancies by direct cytolysis of infected or transformed cells and by secretion of potent immune mediators [7]. Human gut-associated lymphoid tissues harbour various NK cell subsets, which are certainly involved in maintaining homeostasis between the intestinal microbiota and the mucosal immune system [17]. In addition, a human NK-like cell subset expressing NKp44 and IL-22 but lacking classic NK cells molecules such as perforin has been more recently identified [18–20]. Gut-associated NK cells might play an important role in mucosal homeostasis and protective immune responses, particularly under microbial challenge.

In addition, although evidence of a direct action of commensal bacteria, including LAB, on NK cells is still elusive, recent studies suggested that LAB-induced DC regulation might affect NK cell activity. It has been reported that DCs matured by LAB consistently induce activation and promote proliferation and cytotoxicity in autologous NK cells, and that strains of different LAB species differ importantly in their capacity to induce IFN-γ production in NK cells via DCs [14].

This review addresses NK/DC interactions in response to gut-derived LAB and the implications of LAB strain-dependent immunomodulatory effects. Finally, we discuss the potential in vivo impact of commensal bacteria on NK/DC interplay in mucosal tissues, with particular regard to the ability of distinct species of commensal bacterial probiotics to differently polarize the adaptive immune response.

2. NK-DC Interactions: Molecular Mechanisms

Several in vitro studies show a central role of DC-derived IL-12, IL-18, and type I IFN in the triggering of NK cell functions. IL-12 seems to be important to induce the secretion of IFN-γ by NK cells in several systems: LPS-activated monocyte-derived DCs, splenic DCs [21, 22], or poly(I:C)-stimulated myeloid DC [22]. IL-18 may act in synergy with IL-12 to induce the secretion of IFN-γ by NK cells but also to enhance cytotoxicity, at least when NK cells are stimulated with human CD34+ derived DCs [23]. Type I IFNs have also been shown to enhance cytotoxicity of NK cells [3, 24]. Although all types of DCs can secrete type I IFN, the main producer of these cytokines are plasmacytoid dendritic cells (pDCs), particularly when activated through TLR7 and TLR9 by virus components [25]. Nevertheless, NK cells may be activated in an IL-12-, IL-18- and type I IFN-independent manner. In fact, DCs from IL-12- and IL-18-deficient mice are able to induce IFN-γ secretion by NK cells. In mice, this capability might be under the control of IL-2 secreted by bone marrow-derived DCs activated by bacterial components [26].

IL-15 produced by mature monocyte-derived DCs appears to be particularly important to stimulate NK cell proliferation. Interestingly, this effect may require the membrane-bound form of IL-15, as the proliferation is abrogated by physical separation of DCs and NK cells [21].

Despite the large mass of data showing the role of soluble factors in NK cells activation, early studies in mice suggest the involvement of cell-to-cell contact [1]. Transwell separation of the two populations could abrogate DC-dependent NK cells' cytotoxicity induction [1]. Contact through an "immunological synapse" may be necessary for the polarized secretion of IL-12 or of other cytokines by DCs toward NK cells [27] as well as for ligand-receptor interaction [28].

Likewise, it is probably through such synaptic formations that NK cells may kill DCs. Several groups have observed that NK cells recognize and lyse monocyte-derived DCs in vitro [2, 29] in a cell-to-cell contact dependent manner. It has been described that NK/DC ratio is a critical factor to induce NK cells-mediated DC death. Whereas a low ratio (1 : 5) leads to DCs maturation, a higher NK/DC ratio (5 : 1) causes killing of immature DCs by the autologous NK cells [29]. Interestingly, DC subsets display different susceptibilities to lysis by NK cells; human pDCs were not lysed by IL-2 activated NK cells whereas mDCs isolated directly from blood underwent only a limited lysis [22].

Moreover, mature DCs are protected from NK cell lysis by acquiring a higher expression of HLA I molecules [30]. Beside the inhibitory receptors, NK cells activating receptors play a primary role in DC targeting. The activating receptor NKp30 appears to be an important candidate during this interaction, since the single blocking of this receptor inhibits NK cell-mediated lysis of immature DCs [2].

In peripheral tissues, the bidirectional crosstalk between NK cells and DCs has been proposed to play a relevant role in the mechanisms leading to the selection of DCs with maximal capability of T cell priming [4, 31]. In particular, distinct studies have demonstrated that human NK cells have the capability to induce DC maturation [22, 29, 32]. This might be important when pathogen-related molecules or inflammation are not present to drive DC maturation and, therefore, an effective antigen presentation.

The molecular mechanisms that regulate this specific part of the human NK/DC crosstalk have been also clarified. It has been found that, at low NK/DC ratio, NK-DC interactions induces cytokine production (especially, TNF-α and IL-12) by DCs as well as the upregulation of a series of molecules involved in antigen presentation. This stimulating effect may depend on cell-to-cell contact as well as TNF-α released by NK cells [29, 32].

3. Crosstalk between NK Cells and Plasmacytoid DCs

The crosstalk of NK cells with pDCs has not been investigated as much as with myeloid DCs. Remarkably, NK cells and pDCs share different specific receptors and are thus likely to respond to similar stimuli and possibly to be involved in the same phases of the innate immune response [33, 34].

Cytolytic activity of NK cells has long been known to be enhanced by IFN-α [35], and pDCs, also known as type I interferon-producing cells [36, 37], have been shown to be

required for NK cell-mediated lysis of virus-infected target cells [38].

In humans, the pDC pattern of TLR expression is profoundly different from that of myeloid DCs. pDCs do not express TLR1, 2, 3, 4, 5, or 6 but express TLR7 that recognizes viral RNA and TLR9 that recognizes CpG-rich unmethylated DNA from bacteria and DNA viruses. Signaling by TLRs expressed on pDCs drives the production of type I IFN that can directly activate antiviral responses and augment both innate and adaptive immunity to viral as well as nonviral infections [25]. Similar to NK cells [39], pDCs express TLR9. Because both NK cells and pDCs express TLR9, under appropriate conditions, they can potentially be activated by the same invading pathogen simultaneously. The abundant release of type I IFN by pDCs [40], stimulated through TLR9 [41], suggests that the NK cells/pDCs interaction can result in enhanced antiviral innate protection. Through TLR9, the NK/pDC interaction results in upregulation of NK cell-mediated cytotoxicity against various tumor cell targets [3, 24, 42]. This effect is strongly reduced by antibodies against IFN-α, thus indicating a primary role for this cytokine in regulating NK cell functions [24]. Indeed, a sharp up-regulation of CD69 surface expression, confirmed that, under these conditions, NK cells become activated. However, only the small $CD56^{bright}$ NK cell subset can proliferate in the presence of pDCs and TLR9 ligands [24]. Nevertheless, pDC-induced NK cell proliferation appears to be IL-15-independent since, differently from monocyte-derived DCs [21], surface IL-15 is not detectable in TLR-stimulated pDCs [43]. In turn, NK cells are capable of promoting pDC maturation and of up-regulating their production of IFN-α in response to CpG [22]. It is of note that, although NK cells cannot exert an editing program on pDCs, owing to the poor susceptibility of these cells to NK cell-mediated lysis, when cocultured with TLR9-stimulated pDCs, NK cells acquire lytic activity against monocyte-derived immature DCs [24].

4. Lactic Acid Bacteria and Probiotics

The gastrointestinal tract constitutes an important interface between host and environment and, as such, has the dual role of excluding pathogens while facilitating the absorption of nutrients. It is colonized by an estimated 10^{14} microbes, with the density of colonization increasing from the stomach to the distal colon. Commensal bacteria participate in both tasks of the gastrointestinal tract: some help in the absorption of otherwise indigestible nutrients, especially complex carbohydrates, and some contribute to colonization resistance, that is, the ability to inhibit colonization or overgrowth of allegedly pathogenic microorganisms by (i) producing antimicrobial substances, (ii) competing for adhesion sites and nutrients, and (iii) by stimulating the immune system.

Lactic acid bacteria (LAB) are a group of commensal bacteria characterized by their main metabolite and comprise bacteria belonging to several genera (*Lactococcus, Streptococcus, Enterococcus, Leuconostoc,* and *Lactobacillus*). Moreover, the genus *Bifidobacterium* is often mentioned in LAB contexts, although the main fermentation product of bifidobacteria is acetic acid. LAB (hereafter, comprising bifidobacteria) are predominantly nonpathogenic, Gram positive, catalase negative, nonsporeforming, facultative anaerobe, and obligate fermentative. These bacteria have traditionally been applied in food fermentations due to their lactic acid production contributing acidity and thereby prolonged conservation to the foodstuff, and to the pleasant flavor of other metabolites [44]. Some strains of lactobacilli and bifidobacteria are acid and bile tolerant and can be isolated from the mammal gastrointestinal system. In the stomach and in the upper part of the small intestine, lactobacilli outnumber other bacteria whereas bifidobacteria are mainly present in the anaerobic colon, as the dominant genus in breast-fed infants and in adulthood coexisting with many other bacterial strains [45]. As a consequence, food containing viable LAB, such as yoghurt, has gained a reputation of benefiting intestinal well-being, and LAB strains with assumed health effects have been termed "probiotic" (prolife, as opposed to antibiotic). The definition of probiotics currently employed by the World Health Organization is "live microorganisms, which when administered in adequate amounts, confer a health benefit on the host" [46].

"Probiotic" can also designate other microorganisms, for instance certain strains of *E. coli* and *Saccharomyces cerevisiae*, but the majority of commercial probiotics are LAB [47]. The effect of LAB on intestinal health can be assigned to their ability to competitively exclude pathogenic bacteria, to degrade indigestible food components (i.e., fibre or lactose in lactose-intolerant individuals) to valuable nutrients, and finally to support epithelial cell survival and intestinal wall integrity [48].

Data from studies in gnotobiotic, that is, noncolonized rodents and rodents with a controlled microbiota, however, suggest that LAB and other non-pathogenic, so-called commensal, gut bacteria enter in a complex relationship with their animal host, benefiting health also via interactions with both the intestinal and the systemic immune system. If the immune system is not stimulated by gut bacteria after birth it does not develop completely, comprises fewer T cells (including Tregs) [49, 50], dendritic cells [51], and generates weaker antibody responses [52]. Interestingly, specific recognition of commensal microorganisms occurs only in the gut and in the mesenteric lymph nodes [53], and this microbial stimulation might skew the immune system away from the neonatally dominating Th2 response towards a balanced Th1 immune profile [54].

5. LAB as Immunomodulators

Individual species of LAB bacteria possess varying immunomodulatory properties and may finely polarize the immune response. LAB mainly exert direct effects on antigen presenting cells such as dendritic cells, monocytes, macrophages, and, to a minor extent, B-cells, without necessarily being the source of antigen. Cytokines and maturation markers induced by LAB stimulation on APC are, together with the type of antigen encountered, determinants of an ensuing T-cell response [55]. Comparison of stimulation of monocytes/macrophages with LAB as opposed to Gram-negative

bacteria has shown that LAB preferentially induce IL-12 production whereas Gram-negative bacteria such as *E. coli* mainly induce IL-10 [56, 57].

Gastrointestinal-associated lymphoid tissue (GALT) harbours two subset of DCs: myeloid DCs (mDCs), expressing CD11c, and plasmacytoid DCs (pDCs), lacking CD11c and expressing BDCA-2 and ILT-7 [58]. Since myeloid and plasmacytoid DCs express different repertoires of TLRs, they are differently involved in the detection of various pathogens [59]. LAB stimulation of mDCs yields a complicated picture, as different strains of the same genus or even species of LAB can induce different amounts of IL-10 and IL-12. In addition to these Th1-Th2/Treg polarizing cytokines, LAB stimulation frequently leads to TNF-α and IL-6 production by DCs [60, 61]. Concomitantly with induction of cytokine production, distinct LAB induce different levels of maturation in DCs. Generally, LAB induce "semimature DCs", which express lower levels of CD40, CD80, CD86 than DCs matured with pathogenic Gram-positive bacteria and Gram-negative bacteria [62], and induction of maturation markers correlates with their capacity to induce IL-12 and TNF-α. Among gut-derived LAB, *Lactobacillus*-dependent induction of surface markers varies significantly with the strain whereas most bifidobacteria induce low levels of DC maturation [14]. The mucosal microbiota might also modulate IFN-α production by pDCs [63]. Although recent data have shown that LAB do not directly trigger activation of pDCs, *in vitro* studies have suggested a modulation of type I IFN production by the mucosal microenvironment on activated pDCs [63].

How probiotics could modulate activated pDCs remains an interesting field of investigation: it is hypothesized that commensal bacteria may act through specific intracytoplasmic TLRs with the help of humoral immunity or, alternatively, another intriguing scenario may envisage the cooperation of myeloid DCs in LAB-mediated pDC regulation [64].

6. Active Components of LAB

Consensus has not been reached regarding whether bacteria have to survive passage of the gastrointestinal tract to be termed probiotic. However, a picture is emerging where live bacteria are obviously required to reach the gut to metabolize fibres and to outgrow pathogens whereas similar in vitro immunomodulatory effects of LAB can be obtained with both live and dead bacteria [13]. For some immunomodulatory effects, cell integrity is required [65], but in other cases, bacterial components are stimulatory by themselves. Candidate active molecules of LAB are DNA [66, 67] and cell wall components [68, 69]. CpG DNA is a ligand of Toll-like receptor (TLR9) and cell wall peptidoglycan, lipoteichoic acid, and lipopeptides are recognised by TLR2 in conjunction with TLR1 or TLR6 [70]. LAB-ligation of TLRs results in translocation of NFκB to the nucleus with secretion of proinflammatory cytokines (IL-1β, TNFα, and IL-6) as a consequence. IL-12 production is also likely to be a product of TLR-stimulation, as expression of the subunit IL-12p40 in DC in the lamina propria of the small intestine requires binding of NFκB [71], but also intracellular Nucleotide-binding oligomerisation domain (NOD) receptors may recognize LAB peptidoglycan [72, 73]. Regarding the suppressive cytokine IL-10, it can be induced when microbes are recognised by TLR2 or via C-type lectin receptors such as DC-SIGN [55]. It is intriguing that, apparently, small differences in LAB surface structure result in distinct DCs maturation and cytokine induction patterns, which function as a kind of "strain-fingerprint" [74], although for most LAB these mechanisms still remain poorly understood.

7. LAB Shaping NK/DC Crosstalk

Because LAB have a profound modulatory effect on DCs and because DCs in turn are potent activators of NK cells, it is reasonable to expect that DCs that have encountered LAB and undergo maturation can stimulate NK cells. This hypothesis has been recently addressed, and it is now clear that human monocyte-derived DCs [14], blood DCs [75], mouse splenic [76] and lymph node DCs [77] matured by IL-12-inducing LAB activate NK cells to produce IFN-γ, which is in accordance with the belief that IL-12 is essential for IFN-γ production in NK cells. Similarly, LAB-stimulated monocytes produce IL-12 and induce IFN-γ production in NK cells [73–75]. Interestingly, intestine-near mesenteric lymph node NK cells respond to LAB-matured DC with a stronger IFN-γ production than their spleen counterparts [77]. LAB, which do not induce IL-12 in DCs, are equally interesting, because DCs matured by these LAB do not induce IFN-γ production in NK cells, but, added together with IL-12-inducing LAB, they reduce IL-12 production by DCs and thereby IFN-γ production by NK cells. Although the ratio of IL-10/IL-12 induced by non-IL-12-inducing LAB is high, IL-10 is not responsible for this suppression [14]. Rather, LAB with different properties may compete for receptor sites including TLR2 [73]. In addition, when coculturing NK cells with DCs matured by LAB, irrespective of their IL-12-inducing capacities, increased NK cell proliferation and cytotoxicity can be observed [14]. Thus, some LAB might actively contribute to Th1 skewing via the intermediate of NK cells producing IFN-γ whereas a broader panel of LAB strains may increase NK cell number and cytotoxic potential owing to their mutual interaction with dendritic cells (Figure 1).

8. Gut-Derived LAB Induce Species-Dependent IFN-γ Release by NK Cells via DC Activation

In a study comparing three LAB (*L. acidophilus, L. reuteri, and B. bifidum*), only DCs matured by *L. acidophilus* induced high amounts of IFN-γ release by NK cells, suggesting that not all LAB have this capability [14]. It is generally accepted that IL-12 induces IFN-γ production in human NK cells [4]. IFN-γ production by NK cells is required to induce Th1 responses in lymph nodes [78], emphasizing the importance of bacterial regulation of IL-12 production in DCs. Remarkably, it is still not completely elucidated how *L. acidophilus* induces IL-12 production in DCs, although the mechanism involves recognition by TLR2 [73] and phagocytosis [79].

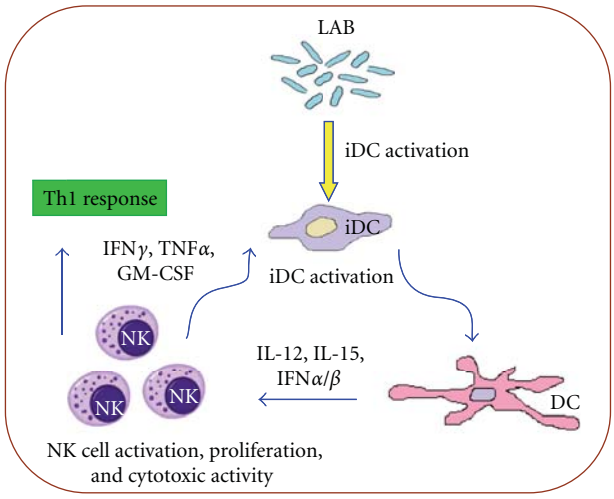

Figure 1: Bi-directional activation between dendritic cell and natural killer (NK) cells in the presence of commensal bacteria. Immature dendritic cells (iDC) are activated and matured by commensal bacteria, for example, lactic acid bacteria (LAB). These LAB-activated mature dendritic cells (DC) produce cytokines able to activate NK cell cytotoxicity and induce their proliferation. Activated NK cells can in turn, via the release of relevant cytokines, recruit (GM-CSF) and activate iDC (TNF-α and IFN-γ). Alternatively, activated NK cells can exert an *editing* of DC by killing some of the iDC. At the same time, the early release of IFN-γ by NK cells interacting with LAB-activated DC, most likely in secondary lymphoid organs such as the mesenteric lymph nodes, is critical for shaping the following adaptive immune response toward a type 1 T cell response. Remarkably, some LAB display opposite outcomes and could hamper T cell type 1 polarization.

This corresponds to Michelsen et al. [80], who showed that maturation and IL-12 production can be induced in murine DCs via TLR2 recognizing purified peptidoglycan and lipoteichoic acid. Of note, IL-12 induction in mDC by strong IL-12-inducing LAB such as *L. acidophilus* has recently been shown to be preceded and partially depend on IFN-β production by the same cells [79]. How this early IFN-β production may affect the DC's interaction with NK cells remains to be investigated.

Being non-IL-12 inducing LAB strains, *B. bifidum* and *L. reuteri* inhibit *L. acidophilus*-induced IL-12 production in DCs and, accordingly, abrogate IFN-γ production by NK cells [14]. This dominant IL-12-inhibitory property of a mixture of LAB may be of importance in the intestine where a large variety of distinct species coexists. The inhibitory components of these bacteria are seemingly soluble and may highlight that such compounds reach gut DC compartments that intact bacteria do not usually access [14, 73].

9. LAB Increasing In Vivo NK Cell Cytotoxic Potential

A number of probiotic LAB, both IL-12-inducing and non-IL-12-inducing strains, have been tested for their ability to increase in vivo NK cell activity as a measure of innate immune activity. Intervention studies have been conducted in healthy volunteers, in which the cytolytic potential of NK cells against standard tumor target cell lines has been measured at several stages of the intervention. Strong evidence of an increase in NK cell activity after probiotic supplementation has been found in elderly individuals [81–83] and in habitual smokers [84]. Often, the increment in NK cell cytolytic activity is lost when probiotic supplementation is terminated [82, 83], reflecting that probiotic bacteria hardly permanently colonize the host. DCs, and not other accessory cells, are likely to be the cell type responsible for the increase in NK cell activity, as DCs present in Peyer's patches and the lamina propria of the intestine have access to the commensal microflora and continuously migrate to mesenteric lymph nodes [84]. In a human study comparing intake of fibre and *L. casei* to reduce recurrence of colon tumors, *L. casei* administration was the preferred intervention [85]. In mice, increased cytolytic potential of NK cells after ingesting probiotic bacteria has been correlated to reduction in tumour incidence [86]. In these studies, however, in addition to a direct effect of NK cells on tumours, NK cells activation may have promoted Th1 polarization and thereby a cytotoxic T cell antitumour response. Investigators have attempted to assign the increase in NK cytolytic activity to an increase in NK cell number or in per-cell cytotoxicity, since both phenomena indeed occur [82, 87].

10. Direct Stimulation of NK Cells by LAB

Direct interaction between LAB and NK cells may occur in the epithelium, where NK cells reside among the intraepithelial lymphocytes [78]. Even if NK cells have previously been shown to be the lymphocyte population most sensitive to activation by LAB, it happens only in the presence of innate accessory cells [88]. These observations, however, do not rule out that NK cells in the gut may be able to directly detect LAB, possibly by interaction between bacterial CpG DNA and TLR9, which is present in NK cells [39]. Studying the direct effect of LAB stimulation on both polyclonally activated and nonactivated NK cells, IFN-γ production was not observed (L.N.F, unpublished data), suggesting that LAB do not interact with TLR9 on NK cells, but rather with TLR2 on accessory cells. TLR2 has been shown to be absent or expressed in low amounts in NK cells [89, 90], and its ligation only activates NK cells in the presence of exogenous IL-12 [91]. On the contrary, induction of cytotoxic activity in NK cells by LAB has been observed both in the presence and absence of accessory cells [92].

To our knowledge, only Yun and colleagues [93] have observed a direct stimulatory effect of gastro-intestinal bacteria on cytokine production by highly pure NK cells using *Helicobacter pylori*. This bacterium may interact with one of the TLRs shown to be functional in NK cells recognizing bacterial products: TLR5 that binds flagellin [94] and TLR9 that detects unmethylated CpG motifs in bacterial DNA [39], and not TLR2, which is expected to be the main receptor involved in the recognition of LAB [95].

11. Role of NK Cells in Th1 Polarization by LAB

Consumption of certain LAB has been shown to alleviate allergy [96, 97]. Matsuzaki and colleagues [98] observed that oral administration of *Lactobacillus casei*, strain *Shirota* (LcS), enhance innate immunity by stimulating the activity of splenic NK cells. Oral feeding with killed LcS was able to stimulate the production of Th1 cytokines, resulting in repressed production of IgE antibodies against ovalbumin in experimental mice. It is believed that LAB skew the allergic Th2 response to a Th1 response via the induction of IL-12 in antigen-presenting cells. Interestingly, NK cells are considered to play a key role in the induction of Th1 responses. It is not known whether NK cells secreting IFN-γ after interaction with DCs participate in Th1 polarization by LAB in vivo. Although the link between in vitro and in vivo activation of NK cells by LAB remains to be established, LAB may become a valuable tool to promote NK activation and thereby Th1 polarization, both as oral adjuvants and as stimulators of DCs exploited in immunotherapy. In addition, the presence of LAB early in life, deflecting the immune system towards a Th1 response, possibly through the intermediate NK cells, may aid in the prevention of Th2-mediated allergy. Finally, the evidence that weak IFN-γ-inducing LAB species are able to suppress the action of IFN-γ-inducing species while preserving NK cell-stimulatory activity could represent a pivotal mechanism in maintaining immunological homeostasis in the intestine or for contrasting Th1-mediated autoimmune diseases, such as Crohn's disease or even celiac disease. Moreover, LAB may represent a useful tool for modulating the cytokine balance during autoimmune diseases driven by Th17 cells in the absence of IFN-γ [99].

12. Concluding Remarks

Commensal bacteria are known to be involved in the maintenance of gut immune homeostasis, and now also emerge as potential NK cell modulators [14]. The crosstalk between NK cells and DCs suggests a critical role for NK cells in the initiation and regulation of immune responses. The considerable knowledge on the molecular basis of these cellular interactions offers opportunities for clinical intervention exploiting DC/NK cell cooperation. For instance, LAB mediate, via DC maturation, proliferation of NK cells and increase of their cytotoxicity. This indicates that LAB, similar to pathogenic microorganisms and inflammatory stimuli, allow DCs to signal to NK cells. Stimulation of NK cell proliferation and cytotoxicity can, therefore, be considered a general ability of LAB. An enlarged and more cytolytic pool of NK cells would be beneficial prophylactically in healthy individuals but also therapeutically in many disease conditions.

Purified NK cells rarely respond to commensal bacteria, and their activation apparently requires the presence of either pDCs or myeloid DCs, depending on the stimulus. A concept emerging from data regarding the crosstalk NK/pDCs is that type I IFN released by pDCs is a potent inducer of NK cell cytotoxicity, suggesting that interaction of NK cells with pDCs can result in enhanced antiviral innate protection. Furthermore, the mucosal microflora might be able to regulate IFN-α production by activated pDCs, although further research is currently highly required in this field of investigation [63, 64].

More generally, the regulatory role of DCs by LAB is of particular importance at mucosal surfaces such as the intestine, where the immune system exists in intimate association with the commensal bacteria [11]. It has been shown that different species of LAB posses the ability to finely regulate myeloid DC maturation and their interactions with NK cells, polarizing the subsequent T cell activity toward Th1, Th2, or even Treg responses [12–14]. This network can, therefore, control not only the strength and the quality of innate responses but also the subsequent adaptive responses, via both cell-to-cell interactions and cytokine release.

In conclusion, LAB potently initiate NK/DC interactions via DC maturation and, as a consequence of that, NK cells increase their cytolytic potential. However, different commensal bacteria have different effects on IFN-γ production by NK cells [14]. Since Th1-promoting LAB or their inhibitory counterpart (non-IL-12-inducing LAB) can easily be identified, these LAB strains may represent a useful tool for modulating the cytokine balance and to promote potent type-1 immune responses, as required in infection and cancer, or to contrast immune dysregulation associated to specific T cell polarization.

All these considerations provide a strong rationale for a combined targeting of NK cells and LAB-stimulated DCs in novel immunotherapeutic strategies, exploiting this cellular crosstalk not only in the treatment of intestinal inflammatory diseases but also in many other conditions for which appropriate immune interventions are required.

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References

[1] N. C. Fernandez, A. Lozier, C. Flament et al., "Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses in vivo," *Nature Medicine*, vol. 5, no. 4, pp. 405–411, 1999.

[2] G. Ferlazzo, M. L. Tsang, L. Moretta, G. Melioli, R. M. Steinman, and C. Münz, "Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells," *Journal of Experimental Medicine*, vol. 195, no. 3, pp. 343–351, 2002.

[3] F. Gerosa, B. Baldani-Guerra, C. Nisii, V. Marchesini, G. Carra, and G. Trinchieri, "Reciprocal activating interaction between natural killer cells and dendritic cells," *Journal of Experimental Medicine*, vol. 195, no. 3, pp. 327–333, 2002.

[4] A. Moretta, "Natural killer cells and dendritic cells: rendezvous in abused tissues," *Nature Reviews Immunology*, vol. 2, no. 12, pp. 957–964, 2002.

[5] M. A. Cooper, T. A. Fehniger, A. Fuchs, M. Colonna, and M. A. Caligiuri, "NK cell and DC interactions," *Trends in Immunology*, vol. 25, no. 1, pp. 47–52, 2004.

[6] G. Ferlazzo and C. Münz, "NK cell compartments and their activation by dendritic cells," *Journal of Immunology*, vol. 172, no. 3, pp. 1333–1339, 2004.

[7] L. Moretta, G. Ferlazzo, C. Bottino et al., "Effector and regulatory events during natural killer-dendritic cell interactions," *Immunological Reviews*, vol. 214, no. 1, pp. 219–228, 2006.

[8] C. E. Andoniou, S. L. H. van Dommelen, V. Voigt et al., "Interaction between conventional dendritic cells and natural killer cells is integral to the activation of effective antiviral immunity," *Nature Immunology*, vol. 6, no. 10, pp. 1011–1019, 2005.

[9] S. Akira and K. Takeda, "Toll-like receptor signaling," *Nature Reviews Immunology*, vol. 4, pp. 499–511, 2004.

[10] J. Banchereau and R. M. Steinman, "Dendritic cells and the control of immunity," *Nature*, vol. 392, no. 6673, pp. 245–252, 1998.

[11] A. J. Stagg, A. L. Hart, S. C. Knight, and M. A. Kamm, "Microbial-gut interactions in health and disease. Interactions between dendritic cells and bacteria in the regulation of intestinal immunity," *Best Practice and Research Clinical Gastroenterology*, vol. 18, no. 2, pp. 255–270, 2004.

[12] H. R. Christensen, H. Frøkiær, and J. J. Pestka, "Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells," *Journal of Immunology*, vol. 168, no. 1, pp. 171–178, 2002.

[13] L. H. Zeuthen, H. R. Christensen, and H. Frøkiær, "Lactic acid bacteria inducing a weak interleukin-12 and tumor necrosis factor alpha response in human dendritic cells inhibit strongly stimulating lactic acid bacteria but act synergistically with gram-negative bacteria," *Clinical and Vaccine Immunology*, vol. 13, no. 3, pp. 365–375, 2006.

[14] L. N. Fink, L. H. Zeuthen, H. R. Christensen, B. Morandi, H. Frøkiær, and G. Ferlazzo, "Distinct gut-derived lactic acid bacteria elicit divergent dendritic cell-mediated NK cell responses," *International Immunology*, vol. 19, no. 12, pp. 1319–1327, 2007.

[15] G. Trinchieri, "Biology of natural killer cells," *Advances in Immunology*, vol. 47, pp. 187–376, 1989.

[16] A. Moretta, C. Bottino, M. Vitale et al., "Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis," *Annual Review of Immunology*, vol. 19, pp. 197–223, 2001.

[17] J. P. Di Santo, C. A. Vosshenrich, and N. Satoh-Takayama, "A 'natural' way to provide innate mucosal immunity," *Current Opinion in Immunology*, vol. 22, no. 4, pp. 435–441, 2010.

[18] M. Cella, A. Fuchs, W. Vermi et al., "A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity," *Nature*, vol. 457, no. 7230, pp. 722–725, 2009.

[19] S. L. Sanos, V. L. Bui, A. Mortha et al., "RORγt and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46+ cells," *Nature Immunology*, vol. 10, no. 1, pp. 83–91, 2009.

[20] C. Luci, A. Reynders, I. I. Ivanov et al., "Influence of the transcription factor RORγt on the development of NKp46+ cell populations in gut and skin," *Nature Immunology*, vol. 10, no. 1, pp. 75–82, 2009.

[21] G. Ferlazzo, M. Pack, D. Thomas et al., "Distinct roles of IL-12 and IL-15 in human natural killer cell activation by dendritic cells from secondary lymphoid organs," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 47, pp. 16606–16611, 2004.

[22] F. Gerosa, A. Gobbi, P. Zorzi et al., "The reciprocal interaction of NK cells with plasmacytoid or myeloid dendritic cells profoundly affects innate resistance functions," *Journal of Immunology*, vol. 174, no. 2, pp. 727–734, 2005.

[23] Y. Yu, M. Hagihara, K. Ando et al., "Enhancement of human cord blood CD34 cell-derived NK cell cytotoxicity by dendritic cells," *Journal of Immunology*, vol. 166, no. 3, pp. 1590–1600, 2001.

[24] C. Romagnani, M. Della Chiesa, S. Kohler et al., "Activation of human NK cells by plasmacytoid dendritic cells and its modulation by CD4 T helper cells and CD4+ CD25+ T regulatory cells," *European Journal of Immunology*, vol. 35, no. 8, pp. 2452–2458, 2005.

[25] M. Colonna, G. Trinchieri, and Y. J. Liu, "Plasmacytoid dendritic cells in immunity," *Nature Immunology*, vol. 5, no. 12, pp. 1219–1226, 2004.

[26] F. Granucci, S. Feau, V. Angeli, F. Trottein, and P. Ricciardi-Castagnoli, "Early IL-2 production by mouse dendritic cells is the result of microbial-induced priming," *Journal of Immunology*, vol. 170, no. 10, pp. 5075–5081, 2003.

[27] C. Borg, A. Jalil, D. Laderach et al., "NK cell activation by dendritic cells (DCs) requires the formation of a synapse leading to IL-12 polarization in DCs," *Blood*, vol. 104, no. 10, pp. 3267–3275, 2004.

[28] Y. M. Vyas, H. Maniar, and B. Dupont, "Visualization of signaling pathways and cortical cytoskeleton in cytolytic and noncytolytic natural killer cell immune synapses," *Immunological Reviews*, vol. 189, pp. 161–178, 2002.

[29] D. Piccioli, S. Sbrana, E. Melandri, and N. M. Valiante, "Contact-dependent stimulation and inhibition of dendritic cells by natural killer cells," *Journal of Experimental Medicine*, vol. 195, no. 3, pp. 335–341, 2002.

[30] G. Ferlazzo, C. Semino, and G. Melioli, "HLA Class I molecule expression is up-regulated during maturation of dendritic cells, protecting them from natural killer cell-mediated lysis," *Immunology Letters*, vol. 76, no. 1, pp. 37–41, 2001.

[31] R. B. Mailliard, Y. I. Son, R. Redlinger et al., "Dendritic cells mediate NK cell help for Th1 and CTL responses: two-signal requirement for the induction of NK cell helper function," *Journal of Immunology*, vol. 171, no. 5, pp. 2366–2373, 2003.

[32] B. Morandi, L. Mortara, P. Carrega et al., "NK cells provide helper signal for CD8+ T cells by inducing the expression of membrane-bound IL-15 on DCs," *International Immunology*, vol. 21, no. 5, pp. 599–606, 2009.

[33] A. Fuchs, M. Cella, T. Kondo, and M. Colonna, "Paradoxic inhibition of human natural interferon-producing cells by the activating receptor NKp44," *Blood*, vol. 106, no. 6, pp. 2076–2082, 2005.

[34] I. Bonaccorsi, C. Cantoni, P. Carrega et al., "The immune inhibitory receptor LAIR-1 is highly expressed by plasmacytoid dendritic cells and acts complementary with NKp44 to control IFNα production," *PLoS One*, vol. 5, no. 11, article 15080, 2010.

[35] G. Trinchieri and D. Santoli, "Antiviral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Enhancement of human natural killer cell activity by interferon and antagonistic inhibition of susceptibility of target cells to lysis," *Journal of Experimental Medicine*, vol. 147, no. 5, pp. 1314–1333, 1978.

[36] B. Perussia, V. Fanning, and G. Trinchieri, "A leukocyte subset bearing HLA-DR antigens is responsible for in vitro

alpha interferon production in response to viruses," *Natural Immunity and Cell Growth Regulation*, vol. 4, no. 3, pp. 120–137, 1985.

[37] F. P. Siegal, N. Kadowaki, M. Shodell et al., "The nature of the principal Type 1 interferon-producing cells in human blood," *Science*, vol. 284, no. 5421, pp. 1835–1837, 1999.

[38] S. Bandyopadhyay, B. Perussia, and G. Trinchieri, "Requirement for HLA-DR+ accessory cells in natural killing of cytomegalovirus-infected fibroblasts," *Journal of Experimental Medicine*, vol. 164, no. 1, pp. 180–195, 1986.

[39] S. Sivori, M. Falco, M. Della Chiesa et al., "CpG and double-stranded RNA trigger human NK cells by toll-like receptors: induction of cytokine release and cytotoxicity against tumors dendritic cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 27, pp. 10116–10121, 2004.

[40] C. Asselin-Paturel and G. Trinchieri, "Production of type I interferons: plasmacytoid dendritic cells and beyond," *Journal of Experimental Medicine*, vol. 202, no. 4, pp. 461–465, 2005.

[41] A. M. Krieg, "CpG motifs in bacterial DNA and their immune effects," *Annual Review of Immunology*, vol. 20, pp. 709–760, 2002.

[42] D. Schepis, I. Gunnarsson, M. L. Eloranta et al., "Increased proportion of CD56 natural killer cells in active and inactive systemic lupus erythematosus," *Immunology*, vol. 126, no. 1, pp. 140–146, 2009.

[43] M. Jinushi, T. Takehara, T. Tatsumi et al., "Autocrine/paracrine IL-15 that is required for type I IFN-mediated dendritic cell expression of MHC class I-related chain A and B is impaired in hepatitis C virus infection," *Journal of Immunology*, vol. 171, no. 10, pp. 5423–5429, 2003.

[44] F. Guarner and G. J. Schaafsma, "Probiotics," *International Journal of Food Microbiology*, vol. 39, no. 3, pp. 237–238, 1998.

[45] H. J. M. Harmsen, A. C. M. Wildeboer-Veloo, G. C. Raangs et al., "Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 30, no. 1, pp. 61–67, 2000.

[46] M. Araya, L. Morelli, G. Reid, M. E. Sanders, and C. Stanton, "Guidelines for the evaluaton of probiotics in food," Report of a Joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food, London, Ontario, Canada, 2002.

[47] W. H. Holzapfel, P. Haberer, J. Snel, U. Schillinger, and J. H. J. Huis In't Veld, "Overview of gut flora and probiotics," *International Journal of Food Microbiology*, vol. 41, no. 2, pp. 85–101, 1998.

[48] A. C. Ouwehand, S. Salminen, and E. Isolauri, "Probiotics: an overview of beneficial effects," *Antonie van Leeuwenhoek*, vol. 82, no. 1–4, pp. 279–289, 2002.

[49] S. Östman, C. Rask, A. E. Wold, S. Hultkrantz, and E. Telemo, "Impaired regulatory T cell function in germ-free mice," *European Journal of Immunology*, vol. 36, no. 9, pp. 2336–2346, 2006.

[50] S. K. Mazmanian, H. L. Cui, A. O. Tzianabos, and D. L. Kasper, "An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system," *Cell*, vol. 122, no. 1, pp. 107–118, 2005.

[51] K. L. W. Walton, J. He, B. L. Kelsall, R. B. Sartor, and N. C. Fisher, "Dendritic cells in germ-free and specific pathogen-free mice have similar phenotypes and in vitro antigen presenting function," *Immunology Letters*, vol. 102, no. 1, pp. 16–24, 2006.

[52] M. V. Herías, C. Hessle, E. Telemo, T. Midtvedt, L. Å. Hanson, and A. E. Wold, "Immunomodulatory effects of Lactobacillus plantarum colonizing the intestine of gnotobiotic rats," *Clinical and Experimental Immunology*, vol. 116, no. 2, pp. 283–290, 1999.

[53] A. J. Macpherson and T. Uhr, "Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria," *Science*, vol. 303, no. 5664, pp. 1662–1665, 2004.

[54] M. Bailey, K. Haverson, C. Inman et al., "The development of the mucosal immune system pre- and post-weaning: balancing regulatory and effector function," *Proceedings of the Nutrition Society*, vol. 64, no. 4, pp. 451–457, 2005.

[55] M. L. Kapsenberg, "Dendritic-cell control of pathogen-driven T-cell polarization," *Nature Reviews Immunology*, vol. 3, no. 12, pp. 984–993, 2003.

[56] M. L. Cross, A. Ganner, D. Teilab, and L. M. Fray, "Patterns of cytokine induction by gram-positive and gram-negative probiotic bacteria," *FEMS Immunology and Medical Microbiology*, vol. 42, no. 2, pp. 173–180, 2004.

[57] C. Hessle, B. Andersson, and A. E. Wold, "Gram-positive bacteria are potent inducers of monocytic interleukin-12 (IL-12) while gram-negative bacteria preferentially stimulate IL-10 production," *Infection and Immunity*, vol. 68, no. 6, pp. 3581–3586, 2000.

[58] J. Schettini and P. Mukherjee, "Physiological role of plasmacytoid dendritic cells and their potential use in cancer immunity," *Clinical and Developmental Immunology*, vol. 2008, Article ID 106321, 10 pages, 2008.

[59] T. T. MacDonald and J. N. Gordon, "Bacterial regulation of intestinal immune responses," *Gastroenterology Clinics of North America*, vol. 34, no. 3, pp. 401–412, 2005.

[60] L. H. Zeuthen, L. N. Fink, and H. Frokiaer, "Epithelial cells prime the immune response to an array of gut-derived commensals towards a tolerogenic phenotype through distinct actions of thymic stromal lymphopoietin and transforming growth factor-β," *Immunology*, vol. 123, no. 2, pp. 197–208, 2008.

[61] M. Mohamadzadeh, S. Olson, W. V. Kalina et al., "Lactobacilli active human dendritic cells that skew T cells toward T helper 1 polarization," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 8, pp. 2880–2885, 2005.

[62] V. Veckman, M. Miettinen, J. Pirhonen, J. Sirén, S. Matikainen, and I. Julkunen, "Streptococcus pyogenes and Lactobacillus rhamnosus differentially induce maturation and production of Th1-type cytokines and chemokines in human monocyte-derived dendritic cells," *Journal of Leukocyte Biology*, vol. 75, no. 5, pp. 764–771, 2004.

[63] N. Contractor, J. Louten, L. Kim, C. A. Biron, and B. L. Kelsall, "Cutting edge: peyer's patch plasmacytoid dendritic cells (pDCs) produce low levels of type I interferons: possible role for IL-10, TGFβ, and prostaglandin E in conditioning a unique mucosal pDC phenotype," *Journal of Immunology*, vol. 179, no. 5, pp. 2690–2694, 2007.

[64] D. Piccioli, C. Sammicheli, S. Tavarini et al., "Human plasmacytoid dendritic cells are unresponsive to bacterial stimulation and require a novel type of cooperation with myeloid dendritic cells for maturation," *Blood*, vol. 113, no. 18, pp. 4232–4239, 2009.

[65] H. H. Smits, A. J. van Beelen, C. Hessle et al., "Commensal Gram-negative bacteria prime human dendritic cells for enhanced IL-23 and IL-27 expression and enhanced Th1 development," *European Journal of Immunology*, vol. 34, no. 5, pp. 1371–1380, 2004.

[66] D. Rachmilewitz, K. Katakura, F. Karmeli et al., "Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis," *Gastroenterology*, vol. 126, no. 2, pp. 520–528, 2004.

[67] K. M. Lammers, P. Brigidi, B. Vitali et al., "Immunomodulatory effects of probiotic bacteria DNA: IL-1 and IL-10 response in human peripheral blood mononuclear cells," *FEMS Immunology and Medical Microbiology*, vol. 38, no. 2, pp. 165–172, 2003.

[68] C. Grangette, S. Nutten, E. Palumbo et al., "Enhanced antiinflammatory capacity of a Lactobacillus plantarum mutant synthesizing modified teichoic acids," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 29, pp. 10321–10326, 2005.

[69] T. Chen, P. Isomäki, M. Rimpiläinen, and P. Toivanen, "Human cytokine responses induced by Gram-positive cell walls of normal intestinal microbiota," *Clinical and Experimental Immunology*, vol. 118, no. 2, pp. 261–267, 1999.

[70] H. Heine and E. Lien, "Toll-like receptors and their function in innate and adaptive immunity," *International Archives of Allergy and Immunology*, vol. 130, no. 3, pp. 180–192, 2003.

[71] C. Becker, S. Wirtz, M. Blessing et al., "Constitutive p40 promoter activation and IL-23 production in the terminal ileum mediated by dendritic cells," *Journal of Clinical Investigation*, vol. 112, no. 5, pp. 693–706, 2003.

[72] D. J. Philpott and S. E. Girardin, "The role of Toll-like receptors and Nod proteins in bacterial infection," *Molecular Immunology*, vol. 41, no. 11, pp. 1099–1108, 2004.

[73] L. H. Zeuthen, L. N. Fink, and H. Frøkiær, "Toll-like receptor 2 and nucleotide-binding oligomerization domain-2 play divergent roles in the recognition of gut-derived lactobacilli and bifidobacteria in dendritic cells," *Immunology*, vol. 124, no. 4, pp. 489–502, 2008.

[74] S. R. Konstantinov, H. Smidt, W. M. De Vos et al., "S layer protein A of Lactobacillus acidophilus NCFM regulates immature dendritic cell and T cell functions," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 49, pp. 19474–19479, 2008.

[75] L. N. Fink, L. H. Zeuthen, G. Ferlazzo, and H. Frøkiær, "Human antigen-presenting cells respond differently to gut-derived probiotic bacteria but mediate similar strain-dependent NK and T cell activation," *FEMS Immunology and Medical Microbiology*, vol. 51, no. 3, pp. 535–546, 2007.

[76] S. I. Koizumi, D. Wakita, T. Sato et al., "Essential role of Toll-like receptors for dendritic cell and NK1.1+ cell-dependent activation of type 1 immunity by Lactobacillus pentosus strain S-PT84," *Immunology Letters*, vol. 120, no. 1-2, pp. 14–19, 2008.

[77] L. N. Fink and H. Frøkiær, "Dendritic cells from Peyer's patches and mesenteric lymph nodes differ from spleen dendritic cells in their response to commensal gut bacteria," *Scandinavian Journal of Immunology*, vol. 68, no. 3, pp. 270–279, 2008.

[78] F. Leon, E. Roldan, L. Sanchez, C. Camarero, A. Bootello, and G. Roy, "Human small-iintestinal epithelium contains functional natural killer lymphocytes," *Gastroenterology*, vol. 125, no. 2, pp. 345–356, 2003.

[79] G. Weiss, S. Rasmussen, L. H. Zeuthen et al., "Lactobacillus acidophilus induces virus immune defence genes in murine dendritic cells by a Toll-like receptor-2-dependent mechanism," *Immunology*, vol. 131, no. 2, pp. 268–281, 2010.

[80] K. S. Michelsen, A. Aicher, M. Mohaupt et al., "The role of toll-like receptors (TLRs) in bacteria-induced maturation of murine dendritic cells (DCs)," *Journal of Biological Chemistry*, vol. 276, no. 28, pp. 25680–25686, 2001.

[81] K. Takeda, T. Suzuki, S. I. Shimada, K. Shida, M. Nanno, and K. Okumura, "Interleukin-12 is involved in the enhancement of human natural killer cell activity by Lactobacillus casei Shirota," *Clinical and Experimental Immunology*, vol. 146, no. 1, pp. 109–115, 2006.

[82] H. S. Gill, K. J. Rutherfurd, M. L. Cross, and P. K. Gopal, "Enhancement of immunity in the elderly by dietary supplementation with the probiotic Bifidobactedum lactis HN019," *American Journal of Clinical Nutrition*, vol. 74, no. 6, pp. 833–839, 2001.

[83] H. S. Gill, K. J. Rutherfurd, and M. L. Cross, "Dietary probiotic supplementation enhances natural killer cell activity in the elderly: an investigation of age-related immunological changes," *Journal of Clinical Immunology*, vol. 21, no. 4, pp. 264–271, 2001.

[84] K. Morimoto, T. Takeshita, M. Nanno, S. Tokudome, and K. Nakayama, "Modulation of natural killer cell activity by supplementation of fermented milk containing Lactobacillus casei in habitual smokers," *Preventive Medicine*, vol. 40, no. 5, pp. 589–594, 2005.

[85] H. Ishikawa, I. Akedo, T. Otani et al., "Randomized trial of dietary fiber and Lactobacillus casei administration for prevention of colorectal tumors," *International Journal of Cancer*, vol. 116, no. 5, pp. 762–767, 2005.

[86] J. H. Niess and H. C. Reinecker, "Dendritic cells: the commanders-in-chief of mucosal immune defenses," *Current Opinion in Gastroenterology*, vol. 22, no. 4, pp. 354–360, 2006.

[87] A. Takagi, T. Matsuzaki, M. Sato, K. Nomoto, M. Morotomi, and T. Yokokura, "Enhancement of natural killer cytotoxicity delayed murine carcinogenesis by a probiotic microorganism," *Carcinogenesis*, vol. 22, no. 4, pp. 599–605, 2001.

[88] D. Haller, S. Blum, C. Bode, W. P. Hammes, and E. J. Schiffrin, "Activation of human peripheral blood mononuclear cells by nonpathogenic bacteria in vitro: evidence of NK cells as primary targets," *Infection and Immunity*, vol. 68, no. 2, pp. 752–759, 2000.

[89] M. Muzio, D. Bosisio, N. Polentarutti et al., "Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells," *Journal of Immunology*, vol. 164, no. 11, pp. 5998–6004, 2000.

[90] V. Hornung, S. Rothenfusser, S. Britsch et al., "Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides," *Journal of Immunology*, vol. 168, no. 9, pp. 4531–4537, 2002.

[91] J. Sawaki, H. Tsutsui, N. Hayashi et al., "Type 1 cytokine/chemokine production by mouse NK cells following activation of their TLR/MyD88-mediated pathways," *International Immunology*, vol. 19, no. 3, pp. 311–320, 2007.

[92] S. Cheon, K. W. Lee, K. E. Kim et al., "Heat-killed Lactobacillus acidophilus La205 enhances NK cell cytotoxicity through increased granule exocytosis," *Immunology Letters*, vol. 136, no. 2, pp. 171–176.

[93] C. H. Yun, A. Lundgren, J. Azem et al., "Natural killer cells and Helicobacter pylori infection: bacterial antigens and interleukin-12 act synergistically to induce gamma interferon production," *Infection and Immunity*, vol. 73, no. 3, pp. 1482–1490, 2005.

[94] A. Chalifour, P. Jeannin, J. F. Gauchat et al., "Direct bacterial protein PAMPs recognition by human NK cells involves TLRs

and triggers α-defensin production," *Blood*, vol. 104, pp. 1778–1783, 2004.

[95] H. Karlsson, P. Larsson, A. E. Wold, and A. Rudin, "Pattern of cytokine responses to gram-positive and gram-negative commensal bacteria is profoundly changed when monocytes differentiate into dendritic cells," *Infection and Immunity*, vol. 72, no. 5, pp. 2671–2678, 2004.

[96] M. Kalliomäki, S. Salminen, H. Arvilommi, P. Kero, P. Koskinen, and E. Isolauri, "Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial," *Lancet*, vol. 357, no. 9262, pp. 1076–1079, 2001.

[97] D. Fujiwara, S. Inoue, H. Wakabayashi, and T. Fujii, "The anti-allergic effects of lactic acid bacteria are strain dependent and mediated by effects on both Th1/Th2 cytokine expression and balance," *International Archives of Allergy and Immunology*, vol. 135, no. 3, pp. 205–215, 2004.

[98] T. Matsuzaki and J. Chin, "Modulating immune responses with probiotic bacteria," *Immunology and Cell Biology*, vol. 78, no. 1, pp. 67–73, 2000.

[99] L. E. Harrington, R. D. Hatton, P. R. Mangan et al., "Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages," *Nature Immunology*, vol. 6, no. 11, pp. 1123–1132, 2005.

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Review Article

Role of Common-Gamma Chain Cytokines in NK Cell Development and Function: Perspectives for Immunotherapy

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NK cells are components of the innate immunity system and play an important role as a first-line defense mechanism against viral infections and in tumor immune surveillance. Their development and their functional activities are controlled by several factors among which cytokines sharing the usage of the common cytokine-receptor gamma chain play a pivotal role. In particular, IL-2, IL-7, IL-15, and IL-21 are the members of this family predominantly involved in NK cell biology. In this paper, we will address their role in NK cell ontogeny, regulation of functional activities, development of specialized cell subsets, and acquisition of memory-like functions. Finally, the potential application of these cytokines as recombinant molecules to NK cell-based immunotherapy approaches will be discussed.

1. Background: The Common-Gamma Chain Cytokine Family

Cytokines are soluble mediators of intercellular signals and play an essential role in the activation and regulation of both adaptive and innate immunity. In particular, the family of cytokines, sharing the common cytokine-receptor gamma-chain (γ_c or CD132) in their receptor complexes, consists of several members with a similar four alpha-helix bundle structure. This family comprises interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21, which display functional redundancy in the regulation of the immune response and in the homeostasis of the lymphoid system but have also specific functions [1]. Each of these cytokines binds to a specific high affinity receptor complex formed by a cytokine-specific α chain and the γ_c [2, 3] (Figure 1). Different from other members of this family, IL-2 and IL-15 can bind with high affinity to heterotrimeric receptor complexes, which consist of IL-2Rα (CD25) or IL15-Rα, respectively, and of IL-2Rβ (CD122) and γ_c chains [4, 5]. The γ_c is an essential component of the receptors of all these cytokines, as it associates to the Janus tyrosine-kinase (JAK)-3, which is required for signal transduction [6]. JAK-3 phosphorylates different downstream signal transducer and activator of transcription (STAT) molecules, in relationship to the type of the receptor complex involved (Figure 1). Thus, IL-4 predominantly signals through STAT-6, whereas IL-2, IL-7, IL-9, and IL-15 mainly activate STAT-5, and IL-21 acts through STAT-3 and STAT1 [1, 7]. The activation of different STAT proteins by JAK-3 is related to its ability to phosphorylate the intracytoplasmic tail of different receptor chains. Tyrosine-phosphorylation of aminoacidic motifs in cytokine-specific receptor molecules generates docking sites for specific unphosphorylated STAT monomers, which are recruited to the receptor complex through their SH-2 domains [8]. Upon tyrosine phosphorylation by JAK-3, phosphorylated STAT molecules dimerize and migrate into the nucleus, where they bind to STAT-sensitive regulatory elements and control

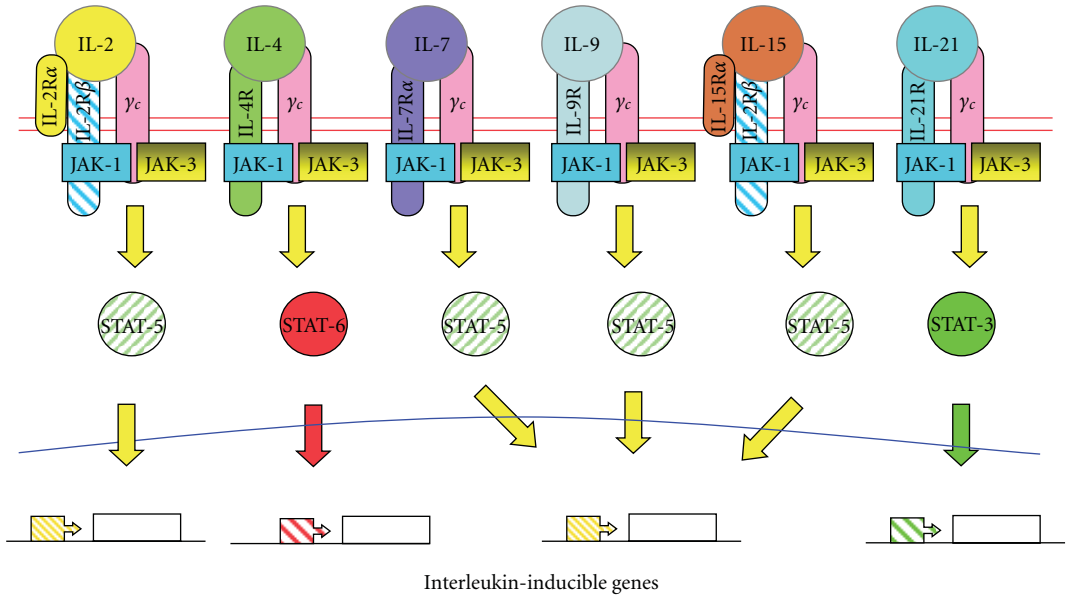

Figure 1: The family of cytokines sharing the common cytokine-receptor γ_c in their receptor complexes. Each cytokine binds to a specific α chain, which forms a receptor complex with the γ_c. In case of IL-2 and IL-15, trimeric high affinity complexes, which include common IL-2Rβ and γ_c chains, can be formed. Each receptor complex mediates signal transduction through JAK1 and/or JAK3 and different STAT molecules. Tyr-phosphorylated STAT dimers regulate transcription of specific cytokine-sensitive genes.

transcription of specific genes. Both the γ_c and JAK3 are essential for the function of all cytokine receptors of this family and are required for the development of the lymphoid cell system. Indeed, genetic defects of γ_c or JAK-3 results in a severe combined immune deficiency (SCID) characterized by the lack of T, B, and NK cells in both mice and humans [9]. In addition to JAK-3, some receptor complexes also activate JAK-1, which predominantly phosphorylates STAT-1 molecules.

IL-2 is the firstly identified member of this cytokine family, and its gene was originally cloned on the basis of the T-cell growth factor (TCGF) activity of this cytokine [10, 11]. Besides its TCGF activity, IL-2 upregulates NK cell proliferation and function, induces lymphokine-activated killer (LAK) activity, and also mediates activated B cell proliferation and Ig production [12]. IL-4 plays an important role in T helper (Th)2 cell development and function, in the regulation of B cell responses, and particularly in IgE production. Therefore, it is involved in allergic diseases and defense against parasitic infections [13]. Few effects of IL-4 have been reported on NK cells. Recent data indicate that IL-4 downregulates the expression of the activating receptor NKG2D in mouse NK cells, thus inhibiting NKG2D-dependent killing in vitro and in vivo [14]. In addition, IL-4 can also inhibit Ly49 receptors expression [15], suggesting a functional role in the innate immunity response. Finally, human NK cells cultured for short term with IL-4 did not release interferon (IFN)-γ and showed no cytolytic activity in response to stimulation through NKp46-activating receptor. In contrast, IL-12-cultured NK cells released IFN-γ and displayed strong cytolytic activity against tumor cells or immature dendritic cells (DC). These data suggest that IL-4 may negatively influence the NK/DC cross-talk, impair Th1 priming, and favor tolerogenic or Th2 responses in humans [16]. Different from the two previous cytokines, which mainly regulate the immune response, IL-7 is fundamental for the homeostasis of the immune system, as it regulates T, B, and NK lymphoid cell development [17]. Indeed, IL-7 is produced by thymic and bone marrow epithelial and stromal cells and by reticular cells in peripheral lymphoid tissues. IL-7 supports differentiation of hematopoietic stem cells into lymphoid progenitor cells and proliferation and survival of lymphoid precursor cells in the bone marrow and in the thymus. In addition, it stimulates survival of naive and memory T cells in the periphery. The crucial role of IL-7 in lymphoid cell development is clearly evidenced by the T and B-cell deficient SCID phenotype of patients and mice with genetic defects of the IL-7Rα (CD127) chain [16, 18]. Although IL-7Rα-deficient patients and mice do not have NK cell deficiency, several data indicate that IL-7 is involved in the development of specific subsets of NK cells [19–24]. IL-9 is a proinflammatory cytokine released by activated $CD4^+$ T cells and mediates activation of eosinophils, mast-cells, and bronchial epithelial cells, thus playing a relevant role in asthma [25]. However, IL-9 appears not involved in NK cell regulation. IL-15, instead, plays a pivotal role in NK cell biology. This cytokine shares several functional activities of IL-2 due to the promiscuous usage not only of the γ_c, but also of the IL-2Rβ chain [4, 12, 26]. However, differently from IL-2, IL-15 is expressed in several tissues and it is produced by different nonlymphoid cell types such as monocytes, DC and stromal cells of the bone marrow and thymus [5]. IL-15 produced in bone marrow, thymus, and secondary lymphoid organs is a crucial element to drive the development and

survival of NK cells [27–29] and of certain subsets of T cells [27, 28]. Finally, IL-21, the most recently identified member of this cytokine family [30] was originally discovered as the ligand of an IL-2Rβ-related orphan receptor [31], now termed IL-21R. IL-21 can costimulate the proliferation of T, B, and NK cells and promotes the terminal differentiation of IL-15 activated NK cells and of activated B cells into plasma cells [32]. In addition, IL-21 mediates apoptosis of partially activated normal B cells [33] and of certain human neoplastic B cells [34–37].

This paper will focus on the role of IL-2, IL-7, IL-15, and IL-21 in NK cell development and function and will discuss the possible relevance of NK activation by these cytokines in cancer immunotherapy.

2. Distinct Role of IL-2 and IL-15 in NK Cell Biology

IL-2 and IL-15 share several functional properties in relationship to the use of two promiscuous receptor components (IL-2Rβ and γ_c) and common signaling pathways. Indeed, both cytokines stimulate the proliferation, survival, and functional activities of NK cells and activated T and B cells. Nonetheless, the two cytokines have also specific functions, which are related to the different cellular distribution and functional properties of the IL-2Rα and IL-15Rα chains and the to the distinct cellular origin and regulation of IL-2 and IL-15 production [12].

IL-2 may act through two types of receptor complexes: the high affinity trimeric receptor formed by IL-2Rα, IL-2Rβ, and γ_c and an intermediate affinity dimeric receptor formed by the IL-2Rβ and γ_c. While the high affinity trimeric receptor is expressed on activated T and NK cells, the intermediate receptor is constitutively expressed on $CD3^{-}CD56^{+}CD16^{+}$ NK cells [38] and on minor subsets of T cells, which can directly respond to high concentrations of IL-2. The constitutive expression of functional IL-2R accounts for the induction of LAK cells, displaying broad non-MHC restricted cytolytic activity against different types of tumor cells by short-term culture of PBMC in IL-2-containing medium [39]. Indeed, LAK cells are predominantly represented by IL-2-activated NK cells and by a subset of T cells [40]. However, a subset of human $CD3^{-}CD56^{bright}CD16^{-}$ NK cells constitutively express high affinity IL-2 receptors and may respond to low IL-2 concentrations [41, 42].

The IL-2 and IL-15 receptor α chains display remarkable differences. IL-2Rα alone has a low affinity for IL-2 and is devoid of signaling properties although it is required for the generation of high-affinity trimeric IL-2R complexes. IL-2Rα expression is induced in T cells upon activation; however, it is constitutively expressed at high levels on immune suppressive $CD4^{+}CD25^{+}FoxP3^{+}$ regulatory T (Treg) cells [43]. Indeed, IL-2 plays a specific role in immune regulation and in peripheral tolerance [44], as it is involved in the maintenance and fitness of $CD4^{+}CD25^{+}FoxP3^{+}$ Treg cells [45, 46]. In addition, IL-2 participates in activation-induced cell death of T cells, which limits T cell responses [47]. The primary role of IL-2 in immune regulation is evidenced by the study of mice defective of IL-2 [48] or of IL-2Rα [49] genes, which develop a lymphoproliferative disorder associated with autoimmunity and impaired Treg function. The genetic defect of the IL-2Rβ chain results in autoimmunity in mice and also in a unique NK-deficient immunophenotype in mice [50] and humans [51]. In addition, an NK cell defect is part of the SCID phenotype in humans [52] and mice [53] bearing genetic defects of the γ_c. Altogether, these studies indicated that IL-2 or IL-2Rα are not necessary for NK cell development, although the IL-2Rβ and the γ_c are strictly required, suggesting a role for IL-15. Indeed, IL-15 and its specific receptor IL-15Rα are essential for the generation and maintenance of NK cells, as IL-15 mediates the development of NK cells from committed NK cell precursors, promotes the differentiation of immature NK cells, and supports the survival of mature NK cells in the peripheral lymphoid organs [54–56].

Different from IL-2Rα, IL-15Rα alone has a high affinity for IL-15 and is constitutively expressed in several lymphoid and nonlymphoid cell types [57, 58]. The study of IL-15- [28] or IL-15Rα-KO [27] mice, which have similar NK-deficient phenotypes, confirmed that the IL-15/IL-15Rα system has an unique essential role in the development and survival of NK cells and of certain subsets of T cells such as NKT cells and intestinal intraepithelial $CD8\alpha\alpha^{+}$ T cells. In addition, IL-15Rα deficient mice have a reduced $CD8^{+}CD44^{high}$ memory T cell pool, indicating a critical role of this receptor in the maintenance of a CD8 memory.

Similar to IL-2, IL-15 can mediate conversion of poorly cytolytic resting NK cells into highly cytolytic effector NK cells, which acquire enhanced antitumor activity [59]. Resting murine NK cells contain abundant granzyme A, but little granzyme B or perforin while the mRNAs for all three genes are highly expressed. IL-2 or IL-15 mediate a dramatic increase in granzyme B and perforin proteins without altering their mRNA abundance. These data suggest that these cytokines can mediate the removal of a block of perforin and granzyme B mRNA translation that prevents resting NK cells to be fully cytotoxic [60].

Several evidences indicate that IL-15 supports NK cell survival [12, 29] and is more potent than IL-2 in this respect. The antiapoptotic effects of IL-15 on murine NK cells are mediated through the inhibition of Bim expression by different mechanisms involving Erk-1/2 phosphorylation or the phosphatidylinositol-3-OH kinase (PI(3)K)-dependent inactivation of the transcription factor Foxo3a. In addition, IL-15 also promotes NK cells survival by the upregulation of Mcl-1, a molecule that was previously reported to be required for the development and survival of T and B lymphocytes [61].

Recent observations indicate that IL-2 or IL-15-activated NK cells display a different sensitivity to corticosteroids. The corticosteroid methylprednisolone inhibited the surface expression of the activating receptors NKp30 and NKp44 and cytolytic activity in IL-2- or IL-15-cultured human NK cells. However, proliferation and survival were inhibited in IL-2- but not in IL-15-cultured NK cells. Moreover, methylprednisolone inhibited activation of STAT-1, STAT-3, and STAT-5 in IL-2-cultured NK cells but only partially in

IL-15-cultured NK cells. This study indicates a distinct ability of IL-15-cultured NK cells to survive to steroid treatment, an observation that is important in immune disorders requiring this drug [62].

Other important differences between IL-2 and IL-15 concern their regulation of expression and secretion. IL-2 gene is transcriptionally activated in Th cells in response to antigen presentation, and then activated Th cell release soluble IL-2, which can activate lymphoid cells expressing high or intermediate affinity receptor complexes. By contrast, IL-15 gene is constitutively expressed in several normal cell types, such as monocytes, DC, stromal cells, epithelial cells and also in some human neoplastic cells [5, 63]. Two IL-15 mRNA isoforms generated by alternative splicing have been identified in human cells: one encodes for a short signal peptide (SSP)-IL-15 and the other for a long-signal peptide (LSP)-IL-15 isoform [64, 65]. The two isoforms show a different intracellular trafficking, as the SSP-IL-15 predominantly localize to the cytoplasm and nucleus, whereas the LSP-IL-15 enters in the endoplasmic reticulum [66]. As a consequence the SSP-IL-15 isoform is not secreted and may have regulatory intracellular functions, whereas the LSP-IL-15, which is usually more expressed, can be potentially exported outside the cell. Nonetheless, it appeared that only very limited amounts of IL-15 are secreted by IL-15 mRNA-expressing cells, possibly in relationship to its low translational rate, to inefficient secretion, and/or to putative retention motifs in the COOH terminus [65–68]. Thus, multiple mechanisms may regulate IL-15 production and secretion.

3. Role of Cytokines in NK Cell Interactions in Secondary Lymphoid Organs

The expression of IL-15 in stromal cells of primary and secondary lymphoid organs and the deficiency of NK cells in mice with genetic defects of the IL-15/IL-15R system indicate a primary role of IL-15 in NK cell development and homeostasis [69–71]. Nonetheless, several data support an important role of a DC/NK cell cross-talk, primarily mediated by cytokines, in the immune response and defense against pathogens in mice [72] and humans [73]. In bacterial infections the interaction between human NK and DC cells may lead to NK cell proliferation, activation, and cytolytic activity [73].

Stimulation in vitro with IL-15, IL-2, IL-1 or IL-18 or engagement of activating receptors (i.e., CD16 or NKG2D) in combination with IL-12 induces production of IFN-γ and of other proinflammatory cytokines and chemokines, such as tumor-necrosis factor (TNF)-α and macrophage inflammatory protein (MIP)-1α by human NK cells [74, 75]. It is of note that cytokines that co-activate NK cells may be produced by monocytes/macrophages or DC and, in this context, it has been shown that DCs colocalize with NK cells in the T cell areas of lymph nodes. Membrane-bound IL-15 is highly exspressed on human DCs activated through CD40 engagement, and is essential for NK cell proliferation and survival. Thus, secondary lymphoid organs are important sites for DC/NK cell interactions [76].

Human $CD3^{-}CD56^{+}$ $NKp46^{+}$ NK cells are a heterogeneous cell population. The $CD56^{dim}CD16^{+}$ cells, predominant in peripheral blood, were originally described as strong cytotoxic effectors with low ability to produce cytokines, by contrast, the $CD56^{bright}CD16^{-}$ NK cell subset, the most represented in secondary lymphoid organs, has been reported to be the most potent producer of IFN-γ but provided of low cytolytic activity [77]. A recent study of De Maria et al. [78] reevaluated the functional capabilities of the two major human NK subsets and showed that NKp46 and NKp30-mediated stimulation induces an early (at 2–4 h from stimulation) and abundant production of IFN-γ by $CD56^{dim}CD16^{+}$ NK cells in relationship to the constitutive expression of IFN-γ mRNA. However, IFN-γ production was transient and no more cytokine production by this cell subset is observed after 16 h from stimulation, when $CD56^{bright}CD16^{+/-}$ cells begin to release IFN-γ. IFN-γ production by the two NK subsets is also observed upon exposure to cytokine combinations such as IL-12+IL-2 or IL-12+IL-15. In this case, $CD56^{dim}CD16^{+}$ NK cells show early but persistent response to stimulation. Therefore, $CD56^{bright}CD16^{+}$ NK cells may play an important role in the early phases of innate responses and in the cross-talk with DC.

Different from $CD56^{dim}$, $CD56^{bright}$NK cells constitutively express the high-affinity heterotrimeric IL-2 receptor complex [41, 42] and can, therefore, respond to picomolar concentrations of IL-2 produced by Th cells. Costimulation of $CD56^{bright}$ human NK cells with IL-2 and IL-12 produced by DC triggers the production of IFN-γ [75]. IFN-γ can further activate DCs and influence the polarization of the Th cell response to Th1. In addition, activated NK cells also produce TNF-α and granulocyte-macrophage colony-stimulating factor (GM-CSF), which support DC differentiation and maturation. Therefore, $CD56^{bright}$ NK cells may link the innate- and antigen-specific immune response, through an NK/DC cross-talk, and shape the adaptive immune response [79, 80].

4. Involvement of Trans-Presentation in IL-15 Activity

The lack of detectable IL-15 secretion, in contrast with its trafficking from the secretory compartment to the early endosomes in some IL-15-expressing human melanoma cells, suggested a possible juxtacrine activity of IL-15. In this early model, IL-15-mediated effects required cell to cell proximity (Figure 2) and could be blocked by an anti-IL-15 monoclonal antibody (mAb) [81]. Further studies showed that IL-15 is expressed as membrane-bound molecule in IFN-γ-stimulated human monocytes, which are capable to stimulate T cell proliferation [82]. In addition, IL-15-mediated proliferation of mouse T cells, triggered by Poly I:C, did not require IL-15Rα on the T cells but instead was dependent on the IL-15Rα present on surrounding cells [83]. These findings could be later explained by the

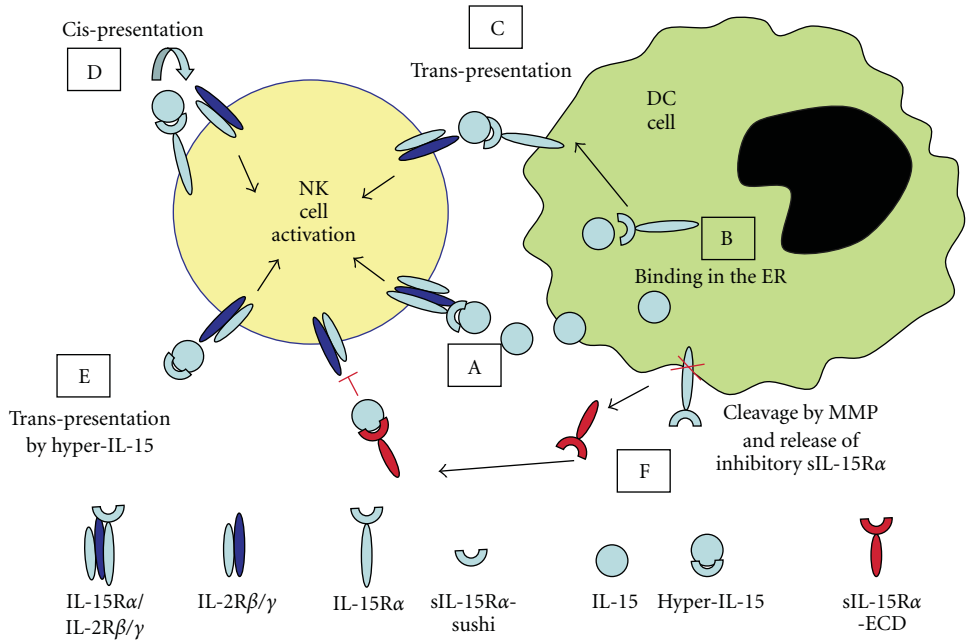

Figure 2: Different mechanisms of IL-15 action. (a): Low levels of secreted IL-15 can bind to high affinity trimeric receptors through a juxtacrine mechanism. (b): IL-15 binds to the high-affinity IL-15Rα chain within the endoplasmic reticulum and is then shuttled to the cell membrane as a complex. (c): IL-15Rα can trans-present IL-15 to an apposing IL-2Rβ/γ_c^+ NK cell through cell contact. (d): The possibility of cis-presentation by IL-15Rα/IL-15 membrane complex to an IL-2Rβ/γ_c heterodimer on the same NK cell is shown. (e): A soluble sushi domain-IL-15Rα bound to IL-15 can perform trans-presentation in a soluble form. (f): Soluble IL-15Rα extracellular domain generated by metalloprotease cleavage can bind soluble IL-15 and block its function.

trans-presentation of IL-15 through IL-15Rα. For trans-presentation, exogenous IL-15 binds to cell surface IL-15Rα chain, the complex is internalized, recycled at the cell membrane and trans-presented to apposing T cells expressing IL-2Rβ/γ_c heterodimers (Figure 2(c)) [84]. Alternatively, endogenously produced LSP-IL-15 binds into the endoplasmic reticulum with IL-15Rα, is exported onto the cell surface of human spleen fibroblasts as a complex with the IL-15Rα chain (Figures 2(b) and 2(c)). The trans-presentation of IL-15 to bystander haematopoietic progenitors commits these cells to the NK differentiation pathway [85]. Similar results have been reported in murine bone marrow DC, where IL-15Rα/IL-15 cell membrane complexes activate NK cells via trans-presentation [86]. The study of in vivo models further supported the role of trans-presentation in peripheral NK cell activation and survival. Indeed, adoptive transfer of normal NK cells into mice lacking the IL-15Rα results in the rapid disappearance of these cells. Conversely, IL-15Rα-deficient NK cells survive upon transfer in normal but not in IL-15Rα-deficient mice. Collectively these data show that IL-15Rα expression on surrounding cells is crucial for the survival of peripheral NK cells, while IL-15Rα expression on NK cells is not involved [87]. The finding that bone marrow progenitors from IL-15Rα$^{-/-}$ mice cultured with IL-7, stem cell factor (SCF) and FMS-like tyrosine kinase 3 ligand (FLT3L), followed by IL-15, differentiate into CD94/NKG2$^+$ NK cells, which lacked Ly49 expression, suggest that IL-15Rα on NK cell precursors is not required for NK cell development but is required for their Ly-49 expression [88].

IL-15 and IL-15Rα have a broad tissue distribution, although their expression is not always coincident, raising the question about the cell type(s) involved in trans-presentation. IL-15 trans-presented by DC has been shown to activate NK cells both in vitro and in vivo [89]. In addition, a study suggested that both hematopoietic cells, such as DC and macrophages and nonhematopoietic cells, including stromal cells and epithelial cells are involved in trans-presentation of IL-15 to human NK cells [90]. Moreover, mice lacking IL-15Rα on macrophages, DCs, or on both, exhibit equivalent defects in NK cell homeostasis and activation, whereas only the expression on macrophages was important for the development of memory CD8$^+$ T cell responses [89].

Besides the relevance of cell-bound IL-15Rα in IL-15 trans-presentation, it has been hypothesized that IL-15Rα may also act as a cis-presenting molecule [83, 91]. In this model, the membrane IL-15Rα/IL-15 complex presents IL-15 to neighbour IL-2Rβ/γ_c complexes on the same cell (Figure 2(d)). In this context, previous data showed that human CD3$^+$ or CD3$^-$ neoplastic lymphoid cells from lymphoproliferative disorder of large granular lymphocytes (LDGL) express surface bound IL-15 [92]. As these cells express IL-15Rα and proliferate to exogenous IL-15 stimulation, it is possible that they may bind IL-15 in vivo and store it on the cell membrane, thus allowing sustained stimulatory effects of endogenous IL-15 through cis-presentation. Nonetheless, the possible source of the in vivo bound IL-15 remains to be determined. The fact that IL-15 may not only

support neoplastic NK cell proliferation but may also play a role in the pathogenesis of LDGL was also suggested by the observation of spontaneous LDGL-like leukemias occurring in mice with transgenic overexpression of IL-15 [93].

In addition to trans- or cis-presentation of IL-15 by IL-15Rα, other forms of membrane-bound IL-15, independent from IL-15Rα, have been described. One of these IL-15 forms has been identified on human monocytes and may behave not only as a ligand but also as a signaling molecule. Thus, membrane-bound IL-15 may produce a reverse signaling that results in cellular adhesion and production of inflammatory cytokines [94]. Also, human haematopoietic progenitors derived from peripheral blood, but not from other sources, express an IL-15Rα-independent membrane-bound IL-15, which mediates reciprocal intercellular signals. This reciprocal trans-presentation induces the in vitro generation of a novel subset of mature noncytolytic NK cells (NKireg) that display regulatory functions and express the immunosuppressive molecule HLA-G. Remarkably, a small subset of NKp46$^+$HLA-G$^+$IL-10$^+$ is detected within freshly isolated decidual NK cells, suggesting that these cells could represent an in vivo counterpart of the in vitro-generated NKireg cells [95]. In addition, these NKireg precursors maintain along their differentiation process in vitro the expression of a membrane-IL-15 able to deliver a bidirectional signal. Indeed, the soluble IL-15Rα chain, upon binding with membrane-IL-15, triggers a reverse signal leading to the appearance of an adherent subset with DC morphology. These cells may represent a terminally differentiated population, since they do not proliferate, display both specific NK (NKp46) and myeloid dendritic (CD1a and BDCA1) markers as well as cytokine production and functions, illustrating another possible chapter of the NK/DC functional interplay [96]. Nonetheless, the in vivo significance of these cells has not yet been explored.

Recent evidences also indicate that soluble forms of IL-15 receptors can be generated by either alternative splicing or by in vivo cleavage of surface IL-15Rα through metalloproteinase-driven mechanisms [97, 98]. Such natural soluble forms of IL-15Rα corresponding to the extracellular domain behave either as a high-affinity IL-15 antagonists (Figure 2(f)) or as superagonist, depending on the isoform involved. Indeed, soluble IL-15R$\alpha\Delta$3 isoform bound to recombinant IL-15 generates a soluble complex, "hyper-IL-15", displaying a 100-fold higher biological activity on IL-2Rβ/γ_c^+ target cells than that exerted by the soluble cytokine [85]. Moreover, a recombinant, soluble sushi domain of IL-15Rα, capable to bind IL-15 with high affinity, is a potent IL-15 agonist and enhances the binding and the biological effects of IL-15 mediated through the IL-2β/γ heterodimer (Figure 2(e)). Nonetheless, the possible in vivo sources of similar "hyper-IL-15" forms and their potential role in the innate and adaptive immune response remains to be determined. In addition, fusion proteins consisting of IL-15 and IL-15Rα-sushi domain linked by a flexible aminoacid sequence (RLI) are even more potent stimulators of NK and T cells [99]. The use of RLI or of soluble IL-15 on a T cell line expressing both IL-15Rα/IL-2Rβ/γ_c trimeric and IL-2Rβ/γ_c dimeric complexes allowed to study the dynamics of cis-presentation (by IL-15) or trans-presentation (by RLI). IL-15 cis-presentation induced fast and transient activation, while trans-presentation mediated slower and more persistent responses [100].

5. Role of Cytokines in NK "Memory-Like" Responses

In specific immunity, the expansion of antigen-specific memory cells and persistent antibody production in response to pathogen's antigen challenge provide enhanced protection against the same pathogen upon a subsequent exposure. By contrast, NK cells are generally thought to be "naturally active" cells, which constitutively display effector functions against infected or transformed cells [101] and are incapable of adapting their responsiveness and of maintaining a memory of a first pathogen encounter in subsequent challenges. However, it is well known that freshly isolated NK cells show low effector functions in terms of cytolytic activity and cytokine production when incubated in vitro with tumor target cells. Several evidences indicate that NK cells can modify their behavior in response to environmental stimuli and can even show memory-like responses in mouse models [102, 103]. For example, recombinase activation gene $(RAG)^{-/-}$ mice (lacking both T and B cells) but not γ_c-deficient mice (lacking also NK cells) can develop delayed-type hypersensitivity (DTH) reactions to hapten. This response was specifically mediated by a subset of liver NK cells expressing Ly49C (an inhibitory receptor for self MHC molecules). In addition, NK cells from sensitized mice develop a specific memory-like function, since their adoptive transfer into naive mice can mediate DTH to the same hapten [104].

Besides this type of memory, which appears to be linked to expression of a specific type of receptor, NK cells may change their way of responding to stimuli following their exposure to specific cytokines [105]. Thus, NK cells from $RAG^{-/-}$ mice, activated for short-term with a cytokine cocktail consisting of IL-12, IL-18, and IL-15, produce high levels of IFN-γ in vitro. Upon subsequent transfer into naïve hosts, these cells can be detected up to three weeks later when they are returned similar to resting NK cells, as they do not display constitutive production of IFN-γ nor enhanced lytic properties. However, they produce significantly more IFN-γ than naïve cells when restimulated. These data suggest that NK cells retain memory of prior cytokine activation. In addition, this memory-like function appeared as an inheritable characteristic, as cytokine-activated NK cells proliferated once injected in mice [105].

As already mentioned, DC can interact with NK cells and activate them via trans-presented IL-15 in vitro [86]. Subsequent findings in a mouse model indicate that similar to T cells, NK cells need to be primed by contact with DC to achieve a full ability to respond to pathogen in vivo and this priming is mediated by IL-15 [106]. Upon engagement of Toll-like receptor by pathogen ligands in the periphery, NK cells migrate into regional lymph nodes where their interact with DCs. NK cell priming required IFN-mediated activation

of CD11c$^+$ DCs, which subsequently trans-present IL-15 to NK cells. Thus priming of NK cells results in arming, as IL-15 can confer full cytolytic properties to NK cells [60]. NK cells become then effector cells and migrate the periphery, where they can efficiently respond to pathogens. Priming and arming is a short-term response finalized to effector functions needed to eliminate pathogens. However, it has been proposed that upon priming and arming NK cells can further develop into memory-like NK cells, which do not produce cytokines or display lytic properties but maintain the capacity to respond to a subsequent pathogen challenge in a more vigorous way than naïve NK cells [102].

Thus, several evidences suggest that NK cells are capable to mediate memory-like responses in different murine models, although the relevance of such responses in humans is still to be defined.

6. Role of IL-7 in NK Cell Subset Development

In addition to IL-15, which is strictly required for the generation of all NK cells [21, 28], other cytokines have been involved in the development of specific NK cell subsets in different organs. In fact, it is clear that NK cells derive from CD34$^+$ hematopoietic progenitor cells originating in the bone marrow. However, early NK precursors can migrate to different organs, where they differentiate towards mature NK cells. Thus, the development of NK cells takes place not only in the bone marrow, but also in the thymus [107, 108], in secondary lymphoid organs [109] and in mucosae-associated lymphoid tissue in the gut in mice and humans [23, 110–112]. Although most mature circulating NK cells do not express IL-7Rα, experimental evidences have been provided that thymic murine NK1.1$^+$ cells express IL-7Rα and that IL-7 is required for the homeostasis of these thymic NK cells [108]. Indeed, differently from classical NK cells, the development of thymic-derived NK cells is dependent upon IL-7 and GATA-3 transcription factor [108]. This peculiar subset is characterized by low cytotoxic activity but high cytokine secretion potential.

A subset of CD56$^+$NKp44$^+$ cells has been identified in human tonsils, and mucosae-associated lymphoid tissues of the gut. Since these cells secrete IL-22 in response to IL-23, they were termed "NK-22" cells [112]. Similar to Th17 cells, NK-22 cells express the transcription factors retinoic-acid-related orphan receptor RORγt and aryl hydrocarbon receptor but do not produce IL-17. IL-7 supports the NK-22 cell survival and maintains the ability to secrete IL-22 in response to IL-23 stimulation. In addition, the combination of IL-7 with IL-1β or IL-2 also mediates NK-22 proliferation, indicating a synergistic effect of IL-7 with these cytokines [22]. The use of IL-1β and/or IL-2 altered the cytokine profile of NK22 cells, suggesting their functional plasticity. Indeed, IL-1β induced constitutive IL-22 secretion, while IL-2 reduced secretion of IL-22 and induced production of IFN-γ.

A murine equivalent of NK-22 cells has been identified in gut-associated lymphoid tissues [20] on the basis of their expression of the natural cytotoxicity receptor (NCR) NKp46, a specific marker of NK cells from several species [113]. This subset was phenotyically characterized as NKp46$^+$IL-7Rα^+RORγt$^+$ and depends for its development on IL-7, RORγt, and intestinal microbial flora [20, 110]. Different from classical NK cells, the development of these mucosal-associated NK cells does not require on IL-15 or IL-2Rβ. Altogether, these data suggest that IL-22–producing NKp46$^+$IL-7Rα^+RORγt$^+$ cells and classical NK cells develop through different pathways under the control of different cytokines [20].

Since intestinal mouse CD3$^-$NKp46$^+$ and human NK-22 cells have some features of immature NK cells, they may possibly represent NK cell precursors that develop locally into specialized NK cell subsets, under the influence of a specific cytokine milieu and microbial product stimulation [23, 111]. In addition, a population of IL-22-producing immature NK cells showing a CD34$^-$IL-7Rα^+CD161$^+$CD9$^-$ surface phenotype, which do not produce IFN-γ and lack of cytolytic activity, has been described in human secondary lymphoid organs [114].

A recent report showed that rare human CD34$^+$ hematopoietic progenitors develop into NK cells in vitro in the presence of cytokines, such as IL-7, IL-15, SCF, and FLT3L. Moreover, the addition of hydrocortisone and stromal cells enhanced the frequency of progenitor cells that could develop into killer cell Ig-like receptor (KIR)$^+$NK cells [115]. These data suggest that NK cells can be derived from precursor cells committed to the myeloid lineage. This latter point is also supported by the existence of human CD14$^+$ myeloid-like cells within cord blood behaving as a novel progenitor for NK cells. Indeed, this CD14$^+$ myeloid-like subset can be redirected into NK differentiation in the presence of IL-15 and then generates mature functional NK cells [116].

CD34$^+$ hematopoietic precursors in human decidua were recently found to express IL-2Rβ, IL-7Rα, and mRNA for E4BP4 and ID2 transcription factors involved in NK cell development [117]. These data suggested that decidual CD34$^+$ cells are precursors committed to the NK cell lineage. In fact, these cells differentiate into functional IL-8- and IL-22-producing CD56^{bright}CD16$^-$KIR$^{+/-}$ NK cells in the presence of growth factors (including IL-15 and IL-7) or upon coculture with decidual stromal cells.

7. IL-21 as a Regulator of NK Cell Responses

IL-21 is produced by CD4$^+$ T cells in response to antigen presentation by DC during the adaptive immune response. At this stage, murine NK cells have been already activated by IL-12 and IL-15 produced by DC and then IL-21 can further support their proliferation and induce their functional maturation into potent effector cells with large granular lymphocyte morphology [118]. Thus, IL-21 upregulates the expression of CD16, the Fc-γRIII required for in antibody-dependent cellular cytotoxicity (ADCC), costimulates the secretion of IFN-γ, and upregulates the expression of granzyme and perforins. Similarly, IL-21 potentiates human NK cell ADCC activity and their ability to secrete cytokines

in response to antibody-coated tumor target cells [119]. IL-21 is also capable to boost NK-mediated ADCC in NK cells with defective cytotoxic properties, such as those of head-and-neck cancer patients [120]. However, different from IL-2 and IL-15, IL-21 alone does not mediate NK cell proliferation though at low concentrations, it costimulates the mitogenic effect of IL-2 or IL-15 [121, 122]. Although it is clear that IL-21 is an important regulator of NK cell functions [30], IL-21 may display positive and negative effects on NK cells, in relationship to their activation/maturation stage and species of origin. In fact, remarkable differences in IL-21 activity have been observed in mouse and human NK cells. Although IL-21 costimulates several functional properties of IL-15-activated murine NK cells, such as IFN-γ production and cytotoxicity, it does not support their survival. Instead, IL-21, at high concentrations, limits the proliferation of NK cells mediated by IL-15 and promotes an apoptotic program. Thus, it has been proposed that IL-21 mediates the transition of an early innate towards an adaptive response in the mouse, through the elimination of terminally differentiated NK cells and the induction of cytotoxic T lymphocyte (CTL) memory responses [123]. Differently, IL-21 alone stimulates the cytolytic activity of freshly isolated, peripheral human NK cells and the combination of low concentrations IL-21 plus IL-15 costimulates the expansion of $CD56^+CD16^+$ NK cells, which develop strong effector functions [121]. Possible explanations for these discrepancies may relate not only to species differences but also to the concentrations of IL-21 and IL-15 that were used in the different experiments or on the timing and activation state of NK cells.

NKG2D is an important activation receptor on mouse and human NK cells and triggers cytotoxicity upon engagement with ligands, such as antibodies or cell surface ligands. These effects are mediated through DNAX-activating protein of 10 kDa (DAP10). Data in a mouse tumor model showed that IL-21 is able to enhance tumor rejection through an NKG2D-dependent mechanism [124] as NKG2D-blocking inhibited the antitumor activity and cytotoxicity of IL-21 activated NK cells. On the opposite, treatment of human NK and $CD8^+$ T cells with IL-21 in combination with IL-2 reduces the cell surface expression of NKG2D and its ability to trigger cytotoxicity, relative to cells treated with IL-2 alone [125]. IL-21-induced downregulation of NKG2D is related to inhibition of DAP10 gene transcription. However, IL-21 induced the expression of the NK activation receptors NKp30 and 2B4, suggesting that IL-21 modulates human NK cell functions and their target specificity by altering the expression levels of different activation/costimulatory receptors.

IL-21 also induced an accelerated development of NK cells from human cord blood $CD34^+$ haematopoietic progenitor cells, in concert with IL-7, IL-15, and SCF. Indeed IL-21 costimulates the expression of NKp46 and NKp30 triggering receptors, CD94/NKG2A inhibitory receptor, KIRs, CD2, and CD16, typical of mature NK cells and the acquisition of cytotoxic activity [126]. In addition, also rare $CD34^-$lineage$^-$ cells cultured with Flt3-L, SCF proliferated in response to IL-15 and IL-21 and acquired a $KIR^-CD56^+CD16^{-/+}$ lymphoid phenotype, consistent with pseudomature NK cells. These cells secreted IFN-γ, GM-CSF and MIP-1α, and displayed cell surface CD107a upon contact with NK-sensitive targets [127]. Thus IL-21 may possibly have a role in the development of NK cells although the study of IL-21 KO mice indicated that IL-21 is not strictly necessary for the differentiation of NK cells from progenitors [56].

8. Perspectives for Cancer Immunotherapy

IL-2 represents a milestone in the history of the immunotherapy of cancer and is still clinically used for the treatment of advanced melanoma and renal carcinoma. The induction of some long-lasting remissions in metastatic patients treated with recombinant (r)IL-2 alone or in combination with LAK cells provided an important proof of principle that activation of the immune system may result in tumor rejection even in patients with bulky disease [128]. However, these effects were observed in a minor subset of patients and the treatment showed a remarkable toxicity, predominantly related to "vascular leak syndrome" [129] and adverse effects on the heart [128]. The availability of novel recombinant cytokines may offer new possibilities for cancer immunotherapy [130, 131].

The functional properties of IL-15 have suggested the use for this cytokine in tumor immunotherapy [12]. It is hoped that IL-15 may display lower toxic effects than IL-2 and provide similar immune-enhancing effects on tumor-reactive T and NK cells. In early experiments, tumor cells transduced with a modified IL-15 cDNA, allowing enhanced IL-15 secretion, showed reduced tumorigenic potential in immunodeficient [132] or in syngeneic mice [133], through the recruitment of NK cells and/or CTLs. In addition, IL-15-transduced tumor cells, administered as vaccine, reduced the incidence of experimental metastases in syngeneic mice. Administration of rIL-15 has also been shown to display antitumor activity in murine tumor models with a lower toxicity than rIL-2 [134]. Finally, plasmid gene transfer of IL-15 trough an hydrodynamic method increases the number and function of NK and IFN-producing killer DC cells in mice [135].

In a preclinical study, human rIL-15, administered intravenously daily for 12 days to rhesus macaques, showed both short- and long-lasting effects on lymphoid cell homeostasis. A transient lymphopenia preceded a clearcut increase in NK and memory $CD8^+$ T cells in the peripheral blood. An inverted CD4/CD8 T-cell ratio was observed as result of $CD8^+$ T cell expansion. By day 48, homeostasis appears restored throughout the body, with the exception of the maintenance of an inverted CD4/CD8 ratio in lymph nodes [136]. A phase I study of intravenous rIL-15 in adults with refractory metastatic melanoma and renal cancer has been recently started and is currently recruiting participants. The objectives of this study are the evaluation of the safety and efficacy of rIL-15 and to examine how the body processes the infused cytokine [NCT01021059].

Since the scaling up of IL-15 production for clinical purposes has been technically difficult, the possible usage of hyper IL-15 in clinical settings of cancer immunotherapy can be envisaged. The potential advantages are that hyper-IL-15

would act in lower doses than IL-15, as it is a more potent activator of the immune system than IL-15 on a molar basis. Interestingly, the potential development of hyper-IL-15 in NK-based immunotherapy is also illustrated by data from Kroemer et al. [137] in a skin transplant in $Rag^{-/-}$ mice. Resting NK cells did not reject skin allografts, while hyper-IL-15-stimulated NK cells mediated acute skin allograft rejection in the absence of T and B cells.

The transfer of NK cells is an emerging strategy for cancer immunotherapy, particularly in haematologic neoplasia. A pivotal role in the antileukemic effects of allogeneic NK cell transfer is played by KIRs, which are inhibitory receptors for HLA class I molecules [138]. The engagement of a KIR expressed on the NK cell surface by the appropriate HLA class I allele on a target cell produces an inhibitory signal to the NK cell activation resulting in target cell protection. In T cell-depleted haploidentical hematopoietic stem cell transplantation (haplo-HSCT) donor NK cells may express KIR(s) that do not recognize the HLA-class I alleles present on recipient's cells. In this "KIR-mismatch" setting, these "alloreactive" NK cells efficiently lyse leukemic cells and generate a strong graft versus leukemia effect, which contributes in eradicating residual disease. In addition, alloreactive NK cells eliminate residual host dendritic cells, thus preventing graft-versus-host disease [138].

Because NK cells are a fraction of peripheral blood mononuclear cells the development of methods to produce large numbers of functional NK cells could be useful to optimize NK-based therapies. Coculture of NK cells with K562 leukemia cells, genetically modified to express membrane-bound IL-15 and 41BBL, allowed a 20-fold expansion of $CD56^+CD3^-$ NK cells from peripheral blood but induced no proliferation of T cells. The expanded NK cells were potent effectors against acute myeloid leukemia cells (AML) in vitro and eradicated AML in xenograft models in immunodeficient mice. This method provide a new platform for expanding activated NK cells for cell therapy of cancer [139].

In view of its immune-enhancing functions, also IL-21 has been considered as a good candidate for cancer immunotherapy. In addition, IL-21, differently from IL-2 is unable to mediate the proliferation of activated $CD4^+CD25^+Foxp3^+$ Treg cells in vitro [140] although Treg cells express IL-21R gene. Nonetheless also IL-21 has shown immune regulatory functions related to the induction of IL-10 production, which inhibits the immune response by acting at several levels [141]. Although this may represent a potential drawback, it is almost likely that each immune enhancing cytokine possess its own negative regulatory mechanisms, to prevent exaggerated responses and autoimmune reactivity.

Several studies in murine tumor models has shown that IL-21 is endowed with antitumor properties [142, 143], which can be mediated by NK, T, or B cell-dependent responses, in relationship to the experimental model considered.

Different types of tumor cells, genetically modified to produce IL-21, form small tumors when injected into syngeneic mice and are then eventually rejected by an IL-21-driven immune reaction, which is followed by immunity to tumor antigens [144]. In addition, human pancreatic cancer cells transduced with murine IL-21 gene are rejected when xenografted in immune-deficient mice through activation of NK cells [145]. In a syngeneic model of mammary adenocarcinoma, tumor-released IL-21 induced the local recruitment of both $CD8^+$ and NK cells and the production of IFN-γ and of IFN-γ-dependent CXC chemokines, which mediated local antiangiogenic effects [144]. IL-21-transduced tumor cells were also effective when used as a vaccine to treat metastatic tumors [140, 146]. However, in the mammary adenocarcinoma model, the therapeutic effect was partial and could be synergistically enhanced by targeting $CD4^+CD25^+$ Treg cells by an anti-CD25 mAb [140]. Thus, Treg cells appeared to limit not only CTL- but also NK-mediated responses by tumor-released IL-21. The antitumor effects of IL-21 therapy did not require Th cells, suggesting that IL-21 may bypass the requirement of Th cells for the induction of CTL and NK responses. In a neuroblastoma syngeneic model the efficacy of IL-21-based immunotherapy was even enhanced by transient $CD4^+$ T cell depletion, which also resulted in the elimination of $CD4^+$ Treg cells [147].

Another approach has been the direct gene transfer of IL-21 in vivo through a plasmid-based hydrodynamic system, which results in sustained IL-21 levels and NK-dependent antitumor effects in syngeneic tumor models [148]. The coinjection of IL-15- and IL-21-encoding plasmids in mice bearing lymphoma produced cooperative effects of tumor rejection [149]. Also, the injection of plasmids encoding for an IL-21/IgFc chimeric protein mediated antitumor effects in melanoma-bearing syngeneic mice, through the induction of an NK-mediated response [150]. Other studies combined IL-21 protein administration with antibody treatments. In view of the ability of IL-21 to enhance ADCC activity by NK cells, rIL-21 has been combined with an anti-Her2/neu antibody to treat mice bearing $Her2/neu^+$ tumors. This combination showed a synergistic antitumor effect through an IFN-γ-dependent mechanism [119].

Altogether preclinical studies led to the design of clinical trials in cancer patients. Several clinical studies of rIL-21 monotherapy or combining IL-21 with other drugs are now ongoing in different type of cancers. Phase I studies in melanoma and renal cancer have been already concluded and showed that IL-21 has an acceptable toxicity profile and does not induce vascular-leak syndrome by repeated iv infusion [151, 152] or subcutaneous administration [153]. IL-21 induced increased levels of soluble CD25 and upregulated IFN-γ, perforin, and granzyme B expression in circulating $CD8^+$ T cells and NK cells, indicating cytotoxic lymphocyte activation. By i.v., IL-21 induced a dose-dependent decrease in circulating NK and T cells, followed by a return to baseline in resting periods. Objective responses and disease stabilizations were observed and were also confirmed in an initial phase II clinical study [154], which also suggested an increase in progression-free survival.

Besides immune-enhancing activities, IL-21 mediates apoptosis of specific B cell malignancies such as chronic lymphocytic leukemia [34, 35] and follicular [37] or diffuse large B cell lymphoma [36]. $CD4^+CD25^+$ regulatory T cells are

expanded in solid and hematological malignancies including CLL and the use of IL-21 instead of IL-2 may contribute to limit this expansion [155]. For this reason and for the ability of IL-21 to enhance ADCC activity by NK cells, a phase I trial of rIL-21 combined with the anti-CD20 mAb rituximab has been designed in chemotherapy refractory/relapsed non-Hodgkin's lymphoma. Acceptable toxicity, several objective responses, and disease stabilizations were reported in a preliminary analysis of data [156]. Overall data from early clinical trials indicate that IL-21 warrants further testing, particularly in multimodality therapy regimens for cancer.

IL-21 also plays a role in controlling chronic viral infections and its serum levels were reduced in HIV-infected persons, which display defective NK activity. Two recent reports indicated that IL-21 enhanced viability, HIV-specific ADCC, IFN-γ secretion, and cytotoxic functions of NK cells from HIV-infected persons [157, 158]. In addition, the IL-21-activated NK cells inhibit viral replication in cocultured $CD4^+$ T cells. These data suggest that that IL-21 could represent a potential tool for immunotherapy or as adjuvant for vaccines in HIV-infected patients.

The systemic administration of cytokines at high doses frequently results in toxicities, and the amount of cytokine effectively delivered at the tumor site is generally low. Tumor cells genetically modified to secrete cytokines may elicit potent immune responses upon injection in syngeneic mice, without systemic effects, due to a high local concentration of cytokine. Although cytokine gene transfer procedures have shown some promising effects in easily accessible tumors (such as subcutaneous melanoma metastases) [159], it cannot be easily applied in case of systemic metastases. Another possibility to reduce systemic toxicity and reach high cytokine concentrations at the tumor site is based on the targeted delivery of cytokines, through the generation of fusion proteins formed by a recombinant antibody linked to a cytokine. These antibody/cytokine chimeras, defined as immunocytokines, have shown promising results in animal models [160]. An example of such immunocytokines is L19-IL-2 [161], which was obtained by chimerization of IL-2 with a single-chain human antibody specific for an oncofetal fibronectin isoform of the tumor extracellular matrix. L19-IL-2 was capable to accumulate at sites of neoangiogenesis in tumors, to determine the recruitment of T and NK and to induce tumor regression in both syngeneic and nude mice. Therefore, L19-IL-2 has entered several clinical trials in different types of tumors and a phase I study was recently concluded [NCT01058538] [162]. This study showed that L19-IL-2 can be safely and repeatedly administered in advanced solid tumours and preliminary evaluation suggests clinical activity in patients with metastatic renal carcinoma.

In a murine neuroblastoma model, the therapeutic effect of an immunocytokine consisting of an anti-GD2 antibody linked to IL-2 was dependent on NK cells [163]. In addition, most human neuroblastomas express low levels of HLA class I [164] and express ligands for NCR activating receptors, thus representing potential targets for NK-based therapies [165]. A phase II trial of the humanized anti-GD2 monoclonal antibody linked to human IL-2 (hu14.18-IL2) was recently performed on relapsed/refractory neuroblastoma patients. To explore the role of NK cells in this treatment patients were genotyped for KIR, HLA, and FcR alleles. The presence of a KIR/KIR-ligand mismatch was signficantly associated with response/improvement to immunocytokine, and there was a trend towards a higher response rate in patients with the FcγR2A 131-H/H genotype than other genotypes. These findings are strongly suggestive for a role of NK cells in clinical responses to hu14.18-IL2 cytokine treatment in relapsed/refractory neuroblastoma patients [166].

9. Conclusions

In conclusion, several evidences indicate that IL-2, IL-7, IL-15, and IL-21 play important roles in NK cell biology and that, in spite of some redundancy, each cytokine has clearly distinct functions. In addition, they may differentially act on subset of NK cells, whose phenotypic and functional heterogeneity is now well established. Moreover, these cytokines allow to manipulate and expand NK cells in vitro to generate populations with increased effector functions or to directly boost NK cell activity in vivo. Thus, these cytokines may represent potentially relevant tools for NK-based immunotherapy strategies in diseases such as leukemias, solid tumors, and AIDS. In this context, the development of novel strategies of cytokine targeted delivery or the use of synergistic combinations with antibodies or of different cytokines may offer the possibility to maximize their therapeutic potential.

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References

[1] Y. Rochman, R. Spolski, and W. J. Leonard, "New insights into the regulation of T cells by γ family cytokines," *Nature Reviews Immunology*, vol. 9, no. 7, pp. 480–490, 2009.

[2] T. Takeshita, H. Asao, K. Ohtani et al., "Cloning of the γ chain of the human IL-2 receptor," *Science*, vol. 257, no. 5068, pp. 379–382, 1992.

[3] M. Kondo, T. Takeshita, N. Ishii et al., "Sharing of the interleukin-2 (IL-2) receptor γ chain between receptors for IL-2 and IL-4," *Science*, vol. 262, no. 5141, pp. 1874–1877, 1993.

[4] R. N. Bamford, A. J. Grant, J. D. Burton et al., "The interleukin (IL) 2 receptor β chain is shared by IL-2 and a cytokine, provisionally designated IL-T, that stimulates T-cell proliferation and the induction of lymphokine-activated killer cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 11, pp. 4940–4944, 1994.

[5] K. H. Grabstein, J. Eisenman, K. Shanebeck et al., "Cloning of a T cell growth factor that interacts with the β chain of the interleukin-2 receptor," *Science*, vol. 264, no. 5161, pp. 965–968, 1994.

[6] K. Ghoreschi, A. Laurence, and J. J. O'Shea, "Janus kinases in immune cell signaling," *Immunological Reviews*, vol. 228, no. 1, pp. 273–287, 2009.

[7] D. De Totero, R. Meazza, M. Capaia et al., "The opposite effects of IL-15 and IL-21 on CLL B cells correlate with differential activation of the JAK/STAT and ERK1/2 pathways," *Blood*, vol. 111, no. 2, pp. 517–524, 2008.

[8] J. J. OShea, M. Gadina, and R. D. Schreiber, "Cytokine signaling in 2002: new surprises in the Jak/Stat pathway," *Cell*, vol. 109, supplement 2, pp. S121–S131, 2002.

[9] P. E. Kovanen and W. J. Leonard, "Cytokines and immunodeficiency diseases: critical roles of the γ-dependent cytokines interleukins 2, 4, 7, 9, 15, and 21, and their signaling pathways," *Immunological Reviews*, vol. 202, pp. 67–83, 2004.

[10] T. Taniguchi, H. Matsui, and T. Fujita, "Structure and expression of a cloned cDNA for human interleukin-2," *Nature*, vol. 302, no. 5906, pp. 305–310, 1983.

[11] S. Gillis, M. M. Ferm, W. Ou, and K. A. Smith, "T cell growth factor: parameters of production and a quantitative microassay for activity," *Journal of Immunology*, vol. 120, no. 6, pp. 2027–2032, 1978.

[12] T. A. Waldmann, "The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design," *Nature Reviews Immunology*, vol. 6, no. 8, pp. 595–601, 2006.

[13] S. Romagnani, "Immunologic influences on allergy and the T1/T2 balance," *Journal of Allergy and Clinical Immunology*, vol. 113, no. 3, pp. 395–400, 2004.

[14] J. Brady, S. Carotta, R. P. L. Thong et al., "The interactions of multiple cytokines control NK cell maturation," *Journal of Immunology*, vol. 185, no. 11, pp. 6679–6688, 2010.

[15] F. Gays, K. Martin, R. Kenefeck, J. G. Aust, and C. G. Brooks, "Multiple cytokines regulate the NK gene complex-encoded receptor repertoire of mature NK cells and T cells," *Journal of Immunology*, vol. 175, no. 5, pp. 2938–2947, 2005.

[16] E. Marcenaro, M. Della Chiesa, F. Bellora et al., "IL-12 or IL-4 prime human NK cells to mediate functionally divergent interactions with dendritic cells or tumors," *Journal of Immunology*, vol. 174, no. 7, pp. 3992–3998, 2005.

[17] A. Puel, S. F. Ziegler, R. H. Buckley, and W. J. Leonard, "Defective IL7R expression in TBNK severe combined immunodeficiency," *Nature Genetics*, vol. 20, no. 4, pp. 394–397, 1998.

[18] J. J. Peschon, P. J. Morrissey, K. H. Grabstein et al., "Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice," *Journal of Experimental Medicine*, vol. 180, no. 5, pp. 1955–1960, 1994.

[19] R. G. J. Klein Wolterink, M. E. García-Ojeda, C. A. J. Vosshenrich, R. W. Hendriks, and J. P. Di Santo, "The intrathymic crossroads of T and NK cell differentiation," *Immunological Reviews*, vol. 238, no. 1, pp. 126–137, 2010.

[20] N. Satoh-Takayama, S. Lesjean-Pottier, P. Vieira et al., "IL-7 and IL-15 independently program the differentiation of intestinal CD3−NKp46+ cell subsets from Id2-dependent precursors," *Journal of Experimental Medicine*, vol. 207, no. 2, pp. 273–280, 2010.

[21] C. A. J. Vosshenrich, T. Ranson, S. I. Samson et al., "Roles for common cytokine receptor γ-chain-dependent cytokines in the generation, differentiation, and maturation of NK cell precursors and peripheral NK cells in vivo," *Journal of Immunology*, vol. 174, no. 3, pp. 1213–1221, 2005.

[22] M. Cella, K. Otero, and M. Colonna, "Expansion of human NK-22 cells with IL-7, IL-2, and IL-1β reveals intrinsic functional plasticity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 24, pp. 10961–10966, 2010.

[23] M. Colonna, "Interleukin-22-producing natural killer cells and lymphoid tissue inducer-like cells in mucosal immunity," *Immunity*, vol. 31, no. 1, pp. 15–23, 2009.

[24] P. Vacca, C. Vitale, E. Montaldo et al., "CD34+ hematopoietic precursors are present in human decidua and differentiate into natural killer cells upon interaction with stromal cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 6, pp. 2402–2407, 2011.

[25] R. J. Noelle and E. C. Nowak, "Cellular sources and immune functions of interleukin-9," *Nature Reviews Immunology*, vol. 10, no. 10, pp. 683–687, 2010.

[26] W. E. Carson, J. G. Giri, M. J. Lindemann et al., "Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor," *Journal of Experimental Medicine*, vol. 180, no. 4, pp. 1395–1403, 1994.

[27] J. P. Lodolce, D. L. Boone, S. Chai et al., "IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation," *Immunity*, vol. 9, no. 5, pp. 669–676, 1998.

[28] M. K. Kennedy, M. Glaccum, S. N. Brown et al., "Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice," *Journal of Experimental Medicine*, vol. 191, no. 5, pp. 771–780, 2000.

[29] W. E. Carson, T. A. Fehniger, S. Haldar et al., "A potential role for interleukin-15 in the regulation of human natural killer cell survival," *Journal of Clinical Investigation*, vol. 99, no. 5, pp. 937–943, 1997.

[30] J. Parrish-Novak, S. R. Dillon, A. Nelson et al., "Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function," *Nature*, vol. 408, no. 6808, pp. 57–63, 2000.

[31] K. Ozaki, K. Kikly, D. Michalovich, P. R. Young, and W. J. Leonard, "Cloning of a type I cytokine receptor most related to the IL-2 receptor β chain," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 21, pp. 11439–11444, 2000.

[32] R. Spolski and W. J. Leonard, "Interleukin-21: basic biology and implications for cancer and autoimmunity," *Annual Review of Immunology*, vol. 26, pp. 57–79, 2008.

[33] D. Konforte, N. Simard, and C. J. Paige, "IL-21: an executor of B cell fate," *Journal of Immunology*, vol. 182, no. 4, pp. 1781–1787, 2009.

[34] D. De Totero, R. Meazza, S. Zupo et al., "Interleukin-21 receptor (IL-21R) is up-regulated by CD40 triggering and mediates proapoptotic signals in chronic lymphocytic leukemia B cells," *Blood*, vol. 107, no. 9, pp. 3708–3715, 2006.

[35] A. Gowda, J. Roda, S. R. A. Hussain et al., "IL-21 mediates apoptosis through up-regulation of the BH3 family member BIM and enhances both direct and antibody-dependent cellular cytotoxicity in primary chronic lymphocytic leukemia cells in vitro," *Blood*, vol. 111, no. 9, pp. 4723–4730, 2008.

[36] K. A. Sarosiek, R. Malumbres, H. Nechushtan, A. J. Gentles, E. Avisar, and I. S. Lossos, "Novel IL-21 signaling pathway up-regulates c-Myc and induces apoptosis of diffuse large B-cell lymphomas," *Blood*, vol. 115, no. 3, pp. 570–580, 2010.

[37] D. de Totero, M. Capaia, M. Fabbi et al., "Heterogeneous expression and function of IL-21R and susceptibility to IL-21-mediated apoptosis in follicular lymphoma cells," *Experimental Hematology*, vol. 38, no. 5, pp. 373–383, 2010.

[38] M. Tsudo, C. K. Goldman, K. F. Bongiovanni et al., "The p75 peptide is the receptor for interleukin 2 expressed on large

granular lymphocytes and is responsible for the interleukin 2 activation of these cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 84, no. 15, pp. 5394–5398, 1987.

[39] E. A. Grimm, R. J. Robb, J. A. Roth et al., "Lymphokine-activated killer cell phenomenon. III. Evidence that IL-2 is sufficient for direct activation of peripheral blood lymphocytes into lymphokine-activated killer cells," *Journal of Experimental Medicine*, vol. 158, no. 4, pp. 1356–1361, 1983.

[40] S. Ferrini, S. Miescher, and M. R. Zocchi, "Phenotypic and functional characterization of recombinant interleukin 2 (rIL 2)-induced activated killer cells: analysis at the population and clonal levels," *Journal of Immunology*, vol. 138, no. 4, pp. 1297–1302, 1987.

[41] M. A. Caligiuri, A. Zmuidzinas, T. J. Manley, H. Levine, K. A. Smith, and J. Ritz, "Functional consequences of interleukin 2 receptor expression on resting human lymphocytes. Identification of a novel natural killer cell subset with high affinity receptors," *Journal of Experimental Medicine*, vol. 171, no. 5, pp. 1509–1526, 1990.

[42] A. Nagler, L. L. Lanier, and J. H. Phillips, "Constitutive expression of high affinity interleukin 2 receptors on human CD16 natural killer cells in vivo," *Journal of Experimental Medicine*, vol. 171, no. 5, pp. 1527–1533, 1990.

[43] M. Itoh, T. Takahashi, N. Sakaguchi et al., "Thymus and autoimmunity: production of CD25+CD4+ naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunologic self-tolerance," *Journal of Immunology*, vol. 162, no. 9, pp. 5317–5326, 1999.

[44] T. R. Malek and I. Castro, "Interleukin-2 receptor signaling: at the interface between tolerance and immunity," *Immunity*, vol. 33, no. 2, pp. 153–165, 2010.

[45] R. Setoguchi, S. Hori, T. Takahashi, and S. Sakaguchi, "Homeostatic maintenance of natural Foxp3(+) CD25(+) CD4(+) regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization," *Journal of Experimental Medicine*, vol. 201, no. 5, pp. 723–735, 2005.

[46] J. D. Fontenot, J. P. Rasmussen, M. A. Gavin, and A. Y. Rudensky, "A function for interleukin 2 in Foxp3-expressing regulatory T cells," *Nature Immunology*, vol. 6, no. 11, pp. 1142–1151, 2005.

[47] Y. Refaeli, L. Van Parijs, C. A. London, J. Tschopp, and A. K. Abbas, "Biochemical mechanisms of IL-2-regulated Fas-mediated T cell apoptosis," *Immunity*, vol. 8, no. 5, pp. 615–623, 1998.

[48] B. Sadlack, J. Löhler, H. Schorle et al., "Generalized autoimmune disease in interleukin-2-deficient mice is triggered by an uncontrolled activation and proliferation of CD4+ T cells," *European Journal of Immunology*, vol. 25, no. 11, pp. 3053–3059, 1995.

[49] D. M. Willerford, J. Chen, J. A. Ferry, L. Davidson, A. Ma, and F. W. Alt, "Interleukin-2 receptor α chain regulates the size and content of the peripheral lymphoid compartment," *Immunity*, vol. 3, no. 4, pp. 521–530, 1995.

[50] H. Suzuki, G. S. Duncan, H. Takimoto, and T. W. Mak, "Abnormal development of intestinal intraepithelial lymphocytes and peripheral natural killer cells in mice lacking the IL-2 receptor β chain," *Journal of Experimental Medicine*, vol. 185, no. 3, pp. 499–505, 1997.

[51] K. C. Gilmour, H. Fujii, T. Cranston, E. Graham Davies, C. Kinnon, and H. B. Gaspar, "Defective expression of the interleukin-2/interleukin-15 receptor β subunit leads to a natural killer cell-deficient form of severe combined immunodeficiency," *Blood*, vol. 98, no. 3, pp. 877–879, 2001.

[52] M. Noguchi, H. Yi, H. M. Rosenblatt et al., "Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans," *Cell*, vol. 73, pp. 147–157, 1993.

[53] J. P. DiSanto, W. Müller, D. Guy-Grand, A. Fischer, and K. Rajewsky, "Lymphoid development in mice with a targeted deletion of the interleukin 2 receptor γ chain," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 2, pp. 377–381, 1995.

[54] M. A. Cooper, J. E. Bush, T. A. Fehniger et al., "In vivo evidence for a dependence on interleukin 15 for survival of natural killer cells," *Blood*, vol. 100, no. 10, pp. 3633–3638, 2002.

[55] T. Ranson, C. A. J. Vosshenrich, E. Corcuff, O. Richard, W. Müller, and J. P. Di Santo, "IL-15 is an essential mediator of peripheral NK-cell homeostasis," *Blood*, vol. 101, no. 12, pp. 4887–4893, 2003.

[56] C. A. J. Vosshenrich, T. Ranson, S. I. Samson et al., "Roles for common cytokine receptor γ-chain-dependent cytokines in the generation, differentiation, and maturation of NK cell precursors and peripheral NK cells in vivo," *Journal of Immunology*, vol. 174, no. 3, pp. 1213–1221, 2005.

[57] D. M. Anderson, S. Kumaki, M. Ahdieh et al., "Functional characterization of the human interleukin-15 receptor α chain and close linkage of IL15RA and IL2RA genes," *Journal of Biological Chemistry*, vol. 270, no. 50, pp. 29862–29869, 1995.

[58] J. G. Giri, S. Kumaki, M. Ahdieh et al., "Identification and cloning of a novel IL-15 binding protein that is structurally related to the α chain of the IL-2 receptor," *EMBO Journal*, vol. 14, no. 15, pp. 3654–3663, 1995.

[59] A. M. Gamero, D. Ussery, D. S. Reintgen, C. A. Puleo, and J. Y. Djeu, "Interleukin 15 induction of lymphokine-activated killer cell function against autologous tumor cells in melanoma patient lymphocytes by a CD18-dependent, perforin-related mechanism," *Cancer Research*, vol. 55, no. 21, pp. 4988–4994, 1995.

[60] T. A. Fehniger, S. F. Cai, X. Cao et al., "Acquisition of murine NK cell cytotoxicity requires the translation of a pre-existing pool of granzyme B and perforin mRNAs," *Immunity*, vol. 26, no. 6, pp. 798–811, 2007.

[61] N. D. Huntington, H. Puthalakath, P. Gunn et al., "Interleukin 15-mediated survival of natural killer cells is determined by interactions among Bim, Noxa and Mcl-1," *Nature Immunology*, vol. 8, no. 8, pp. 856–863, 2007.

[62] L. Chiossone, C. Vitale, F. Cottalasso et al., "Molecular analysis of the methylprednisolone-mediated inhibition of NK-cell function: evidence for different susceptibility of IL-2- versus IL-15-activated NK cells," *Blood*, vol. 109, no. 9, pp. 3767–3775, 2007.

[63] R. Meazza, S. Verdiani, R. Biassoni et al., "Identification of a novel interleukin-15 (IL-15) transcript isoform generated by alternative splicing in human small cell lung cancer cell lines," *Oncogene*, vol. 12, no. 10, pp. 2187–2192, 1996.

[64] R. Meazza, A. Gaggero, F. Neglia et al., "Expression of two interleukin-15 mRNA isoforms in human tumors does not correlate with secretion: role of different signal peptides," *European Journal of Immunology*, vol. 27, no. 5, pp. 1049–1054, 1997.

[65] A. Onu, T. Pohl, H. Krause, and S. Bulfone-Paus, "Regulation of IL-15 secretion via the leader peptide of two IL-15 isoforms," *Journal of Immunology*, vol. 158, no. 1, pp. 255–262, 1997.

[66] A. Gaggero, B. Azzarone, C. Andrei et al., "Differential intracellular trafficking, secretion and endosomal localization of two IL-15 isoforms," *European Journal of Immunology*, vol. 29, no. 4, pp. 1265–1274, 1999.

[67] G. Kurys, Y. Tagaya, R. Bamford, J. A. Hanover, and T. A. Waldmann, "The long signal peptide isoform and its alternative processing direct the intracellular trafficking of interleukin-15," *Journal of Biological Chemistry*, vol. 275, no. 39, pp. 30653–30659, 2000.

[68] R. N. Bamford, A. P. DeFilippis, N. Azimi, G. Kurys, and T. A. Waldmann, "The 5' untranslated region, signal peptide, and the coding sequence of the carboxyl terminus of IL-15 participate in its multifaceted translational control," *Journal of Immunology*, vol. 160, no. 9, pp. 4418–4426, 1998.

[69] F. Colucci, M. A. Caligiuri, and J. P. Di Santo, "What does it take to make a natural killer?" *Nature Reviews Immunology*, vol. 3, no. 5, pp. 413–425, 2003.

[70] S. S. Farag and M. A. Caligiuri, "Human natural killer cell development and biology," *Blood Reviews*, vol. 20, no. 3, pp. 123–137, 2006.

[71] M. A. Caligiuri, "Human natural killer cells," *Blood*, vol. 112, no. 3, pp. 461–469, 2008.

[72] N. C. Fernandez, A. Lozier, C. Flament et al., "Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses in vivo," *Nature Medicine*, vol. 5, no. 4, pp. 405–411, 1999.

[73] G. Ferlazzo, B. Morandi, A. D'Agostino et al., "The interaction between NK cells and dendritic cells in bacterial infections results in rapid induction of NK cell activation and in the lysis of uninfected dendritic cells," *European Journal of Immunology*, vol. 33, no. 2, pp. 306–313, 2003.

[74] T. A. Fehniger, M. H. Shah, M. J. Turner et al., "Differential cytokine and chemokine gene expression by human NK cells following activation with IL-18 or IL-15 in combination with IL-12: implications for the innate immune response," *Journal of Immunology*, vol. 162, no. 8, pp. 4511–4520, 1999.

[75] T. A. Fehniger, M. A. Cooper, G. J. Nuovo et al., "CD56bright natural killer cells are present in human lymph nodes and are activated by T cell-derived IL-2: a potential new link between adaptive and innate immunity," *Blood*, vol. 101, no. 8, pp. 3052–3057, 2003.

[76] G. Ferlazzo, M. Pack, D. Thomas et al., "Distinct roles of IL-12 and IL-15 in human natural killer cell activation by dendritic cells from secondary lymphoid organs," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 47, pp. 16606–16611, 2004.

[77] M. A. Cooper, T. A. Fehniger, S. C. Turner et al., "Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset," *Blood*, vol. 97, no. 10, pp. 3146–3151, 2001.

[78] A. De Maria, F. Bozzano, C. Cantoni, and L. Moretta, "Revisiting human natural killer cell subset function revealed cytolytic CD56dimCD16+ NK cells as rapid producers of abundant IFN-γ on activation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 2, pp. 728–732, 2011.

[79] L. Moretta, G. Ferlazzo, C. Bottino et al., "Effector and regulatory events during natural killer-dendritic cell interactions," *Immunological Reviews*, vol. 214, no. 1, pp. 219–228, 2006.

[80] M. A. Cooper, T. A. Fehniger, A. Fuchs, M. Colonna, and M. A. Caligiuri, "NK cell and DC interactions," *Trends in Immunology*, vol. 25, no. 1, pp. 47–52, 2004.

[81] T. Musso, L. Calosso, M. Zucca et al., "Human monocytes constitutively express membrane-bound, biologically active, and interferon-γ-upregulated interleukin-15," *Blood*, vol. 93, no. 10, pp. 3531–3539, 1999.

[82] J. P. Lodolce, P. R. Burkett, D. L. Boone, M. Chien, and A. Ma, "T cell-independent interleukin 15Rα signals are required for bystander proliferation," *Journal of Experimental Medicine*, vol. 194, no. 8, pp. 1187–1193, 2001.

[83] S. W. Stonier and K. S. Schluns, "Trans-presentation: a novel mechanism regulating IL-15 delivery and responses," *Immunology Letters*, vol. 127, no. 2, pp. 85–92, 2010.

[84] S. Dubois, J. Mariner, T. A. Waldmann, and Y. Tagaya, "IL-15Rα recycles and presents IL-15 in trans to neighboring cells," *Immunity*, vol. 17, no. 5, pp. 537–547, 2002.

[85] J. Giron-Michel, M. Giuliani, M. Fogli et al., "Membrane-bound and soluble IL-15/IL-15Rα complexes display differential signaling and functions on human hematopoietic progenitors," *Blood*, vol. 106, no. 7, pp. 2302–2310, 2005.

[86] E. Mortier, T. Woo, R. Advincula, S. Gozalo, and A. Ma, "IL-15Rα chaperones IL-15 to stable dendritic cell membrane complexes that activate NK cells via trans presentation," *Journal of Experimental Medicine*, vol. 205, no. 5, pp. 1213–1225, 2008.

[87] R. Koka, P. R. Burkett, M. Chien et al., "Interleukin (IL)-15Rα-deficient natural killer cells survive in normal but not IL-15Rα-deficient mice," *Journal of Experimental Medicine*, vol. 197, no. 8, pp. 977–984, 2003.

[88] T. Kawamura, R. Koka, A. Ma, and V. Kumar, "Differential roles for IL-15R alpha-chain in NK cell development and Ly-49 induction," *Journal of Immunology*, vol. 171, no. 10, pp. 5085–5090, 2003.

[89] E. Mortier, R. Advincula, L. Kim et al., "Macrophage- and dendritic-cell-derived interleukin-15 receptor alpha supports homeostasis of distinct CD8+ T cell subsets," *Immunity*, vol. 31, no. 5, pp. 811–822, 2009.

[90] N. D. Huntington, N. Legrand, N. L. Alves et al., "IL-15 trans-presentation promotes human NK cell development and differentiation in vivo," *Journal of Experimental Medicine*, vol. 206, no. 1, pp. 25–34, 2009.

[91] S. K. Olsen, N. Ota, S. Kishishita et al., "Crystal structure of the interleukin-15·interleukin-15 receptor α complex: insights into trans and cis presentation," *Journal of Biological Chemistry*, vol. 282, no. 51, pp. 37191–37204, 2007.

[92] R. Zambello, M. Facco, L. Trentin et al., "Interleukin-15 triggers the proliferation and cytotoxicity of granular lymphocytes in patients with lymphoproliferative disease of granular lymphocytes," *Blood*, vol. 89, no. 1, pp. 201–211, 1997.

[93] T. A. Fehniger, K. Suzuki, A. Ponnappan et al., "Fatal leukemia in interleukin 15 transgenic mice follows early expansions in natural killer and memory phenotype CD8+ T cells," *Journal of Experimental Medicine*, vol. 193, no. 2, pp. 219–231, 2001.

[94] G. G. Neely, S. Epelman, L. L. Ma et al., "Monocyte surface-bound IL-15 can function as an activating receptor and participate in reverse signaling," *Journal of Immunology*, vol. 172, no. 7, pp. 4225–4234, 2004.

[95] M. Giuliani, J. Giron-Michel, S. Negrini et al., "Generation of a novel regulatory NK cell subset from peripheral blood CD34+ progenitors promoted by membrane-bound IL-15," *PLoS One*, vol. 3, no. 5, Article ID e2241, 16 pages, 2008.

[96] S. Negrini, M. Giuliani, D. Durali, S. Chouaib, and B. Azzarone, "Membrane-bound IL-15 stimulation on peripheral blood NK progenitors (PB-NKp) leads to the generation

of an adherent subset co-expressing DC and NK functional markers," *Haematologica*, vol. 96, no. 5, pp. 2762–2766, 2011.

[97] V. Budagian, E. Bulanova, Z. Orinska et al., "Natural soluble interleukin-15Rα is generated by cleavage that involves the tumor necrosis factor-α-converting enzyme (TACE/ADAM17)," *Journal of Biological Chemistry*, vol. 279, no. 39, pp. 40368–40375, 2004.

[98] E. Mortier, J. Bernard, A. Plet, and Y. Jacques, "Natural, proteolytic release of a soluble form of human IL-15 receptor α-chain that behaves as a specific, high affinity IL-15 antagonist," *Journal of Immunology*, vol. 173, no. 3, pp. 1681–1688, 2004.

[99] E. Mortier, A. Quéméner, P. Vusio et al., "Soluble interleukin-15 receptor alpha (IL-15R alpha)-sushi as a selective and potent agonist of IL-15 action through IL-15R beta/gamma. Hyperagonist IL-15 x IL-15R alpha fusion proteins," *Journal of Biological Chemistry*, vol. 281, no. 3, pp. 1612–1619, 2006.

[100] H. Perdreau, E. Mortier, G. Bouchaud et al., "Different dynamics of IL-15R activation following IL-15 cis- or trans-presentation," *European Cytokine Network*, vol. 21, no. 4, pp. 297–307, 2010.

[101] R. B. Herberman and H. T. Holden, "Natural cell-mediated immunity," *Advances in Cancer Research*, vol. 27, pp. 305–377, 1978.

[102] M. A. Cooper and W. M. Yokoyama, "Memory-like responses of natural killer cells," *Immunological Reviews*, vol. 235, no. 1, pp. 297–305, 2010.

[103] E. Vivier, D. H. Raulet, A. Moretta et al., "Innate or adaptive immunity? The example of natural killer cells," *Science*, vol. 331, no. 6013, pp. 44–49, 2011.

[104] J. G. O'Leary, M. Goodarzi, D. L. Drayton, and U. H. von Andrian, "T cell- and B cell-independent adaptive immunity mediated by natural killer cells," *Nature Immunology*, vol. 7, no. 5, pp. 507–516, 2006.

[105] M. A. Cooper, J. M. Elliott, P. A. Keyel, L. Yang, J. A. Carrero, and W. M. Yokoyama, "Cytokine-induced memory-like natural killer cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 6, pp. 1915–1919, 2009.

[106] M. Lucas, W. Schachterle, K. Oberle, P. Aichele, and A. Diefenbach, "Dendritic cells prime natural killer cells by trans-presenting interleukin 15," *Immunity*, vol. 26, no. 4, pp. 503–517, 2007.

[107] M. C. Mingari, C. Vitale, C. Cantoni et al., "Interleukin-15-induced maturation of human natural killer cells from early thymic precursors: selective expression of CD94/NKG2-A as the only HLA class I-specific inhibitory receptor," *European Journal of Immunology*, vol. 27, no. 6, pp. 1374–1380, 1997.

[108] C. A. J. Vosshenrich, M. E. García-Ojeda, S. I. Samson-Villéger et al., "A thymic pathway of mouse natural killer cell development characterized by expression of GATA-3 and CD127," *Nature Immunology*, vol. 7, no. 11, pp. 1217–1224, 2006.

[109] A. G. Freud, B. Becknell, S. Roychowdhury et al., "A human CD34(+) subset resides in lymph nodes and differentiates into CD56 natural killer cells," *Immunity*, vol. 22, no. 3, pp. 295–304, 2005.

[110] S. L. Sanos, V. L. Bui, A. Mortha et al., "RORγt and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46 cells," *Nature Immunology*, vol. 10, no. 1, pp. 83–91, 2009.

[111] E. Vivier, H. Spits, and T. Cupedo, "Interleukin-22-producing innate immune cells: new players in mucosal immunity and tissue repair?" *Nature Reviews Immunology*, vol. 9, no. 4, pp. 229–234, 2009.

[112] M. Cella, A. Fuchs, W. Vermi et al., "A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity," *Nature*, vol. 457, no. 7230, pp. 722–725, 2009.

[113] L. Moretta and A. Moretta, "Unravelling natural killer cell function: triggering and inhibitory human NK receptors," *EMBO Journal*, vol. 23, no. 2, pp. 255–259, 2004.

[114] T. Hughes, B. Becknell, S. McClory et al., "Stage 3 immature human natural killer cells found in secondary lymphoid tissue constitutively and selectively express the T17 cytokine interleukin-22," *Blood*, vol. 113, no. 17, pp. 4008–4010, 2009.

[115] B. Grzywacz, N. Kataria, N. Kataria, B. R. Blazar, J. S. Miller, and M. R. Verneris, "Natural killer cell differentiation by myeloid progenitors," *Blood*, vol. 117, no. 13, pp. 3548–3558, 2011.

[116] S. A. Perez, P. A. Sotiropoulou, D. G. Gkika et al., "A novel myeloid-like NK cell progenitor in human umbilical cord blood," *Blood*, vol. 101, no. 9, pp. 3444–3450, 2003.

[117] P. Vacca, C. Vitale, E. Montaldo et al., "CD34+ hematopoietic precursors are present in human decidua and differentiate into natural killer cells upon interaction with stromal cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 6, pp. 2402–2407, 2011.

[118] J. Brady, Y. Hayakawa, M. J. Smyth, and S. L. Nutt, "IL-21 induces the functional maturation of murine NK cells," *Journal of Immunology*, vol. 172, no. 4, pp. 2048–2058, 2004.

[119] J. M. Roda, T. Joshi, J. P. Butchar et al., "The activation of natural killer cell effector functions by cetuximab-coated, epidermal growth factor receptor—positive tumor cells is enhanced by cytokines," *Clinical Cancer Research*, vol. 13, no. 21, pp. 6419–6428, 2007.

[120] M. Watanabe, K. Kono, Y. Kawaguchi et al., "Interleukin-21 can efficiently restore impaired antibody-dependent cell-mediated cytotoxicity in patients with oesophageal squamous cell carcinoma," *British Journal of Cancer*, vol. 102, no. 3, pp. 520–529, 2010.

[121] J. Parrish-Novak, D. C. Foster, R. D. Holly, and C. H. Clegg, "Interleukin-21 and the IL-21 receptor: novel effectors of NK and T cell responses," *Journal of Leukocyte Biology*, vol. 72, no. 5, pp. 856–863, 2002.

[122] J. A. Toomey, F. Gays, D. Foster, and C. G. Brooks, "Cytokine requirements for the growth and development of mouse NK cells in vitro," *Journal of Leukocyte Biology*, vol. 74, no. 2, pp. 233–242, 2003.

[123] M. T. Kasaian, M. J. Whitters, L. L. Carter et al., "IL-21 limits NK cell responses and promotes antigen-specific T cell activation: a mediator of the transition from innate to adaptive immunity," *Immunity*, vol. 16, no. 4, pp. 559–569, 2002.

[124] R. Takaki, Y. Hayakawa, A. Nelson et al., "IL-21 enhances tumor rejection through a NKG2D-dependent mechanism," *Journal of Immunology*, vol. 175, no. 4, pp. 2167–2173, 2005.

[125] S. J. Burgess, A. I. Marusina, I. Pathmanathan, F. Borrego, and J. E. Coligan, "IL-21 down-regulates NKG2D/DAP10 expression on human NK and CD8+ T cells," *Journal of Immunology*, vol. 176, no. 3, pp. 1490–1497, 2006.

[126] S. Sivori, C. Cantoni, S. Parolini et al., "IL-21 induces both rapid maturation of human CD34 cell precursors towards NK cells and acquisition of surface killer Ig-like receptors," *European Journal of Immunology*, vol. 33, no. 12, pp. 3439–3447, 2003.

[127] G. Bonanno, A. Mariotti, A. Procoli et al., "Interleukin-21 induces the differentiation of human umbilical cord blood

CD34-lineagecells into pseudomature lytic NK cells," *BMC Immunology*, vol. 10, Article ID 1471, p. 46, 2009.

[128] M. B. Atkins, M. T. Lotze, J. P. Dutcher et al., "High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993," *Journal of Clinical Oncology*, vol. 17, no. 7, pp. 2105–2116, 1999.

[129] M. Rosenstein, S. E. Ettinghausen, and S. A. Rosenberg, "Extravasation of intravascular fluid mediated by the systemic administration of recombinant interleukin 2," *Journal of Immunology*, vol. 137, no. 5, pp. 1735–1742, 1986.

[130] C. M. Capitini, T. J. Fry, and C. L. Mackall, "Cytokines as adjuvants for vaccine and cellular therapies for cancer," *American Journal of Immunology*, vol. 5, no. 3, pp. 65–83, 2009.

[131] N. M. Fewkes and C. L. Mackall, "Novel gamma-chain cytokines as candidate immune modulators in immune therapies for cancer," *Cancer Journal*, vol. 16, no. 4, pp. 392–398, 2010.

[132] E. Di Carlo, R. Meazza, S. Basso et al., "Dissimilar antitumour reactions induced by tumour cells engineered with the interleukin-2 or interleukin-15 gene in nude mice," *Journal of Pathology*, vol. 191, no. 2, pp. 193–201, 2000.

[133] R. Meazza, P. L. Lollini, P. Nanni et al., "Gene transfer of a secretable form of IL-15 in murine adenocarcinoma cells: effects on tumorigenicity, metastatic potential and immune response," *International Journal of Cancer*, vol. 87, no. 4, pp. 574–581, 2000.

[134] W. Munger, S. Q. Dejoy, R. Jeyaseelan et al., "Studies evaluating the antitumor activity and toxicity of interleukin-15, a new T cell growth factor: comparison with interleukin-2," *Cellular Immunology*, vol. 165, no. 2, pp. 289–293, 1995.

[135] A. Arina, O. Murillo, J. Dubrot et al., "Interleukin-15 liver gene transfer increases the number and function of IKDCs and NK cells," *Gene Therapy*, vol. 15, no. 7, pp. 473–483, 2008.

[136] E. Lugli, C. K. Goldman, L. P. Perera et al., "Transient and persistent effects of IL-15 on lymphocyte homeostasis in nonhuman primates," *Blood*, vol. 116, no. 17, pp. 3238–3248, 2010.

[137] A. Kroemer, X. Xiao, N. Degauque et al., "The innate NK cells, allograft rejection, and a key role for IL-15," *Journal of Immunology*, vol. 180, no. 12, pp. 7818–7826, 2008.

[138] L. Moretta, F. Locatelli, D. Pende, E. Marcenaro, M. C. Mingari, and A. Moretta, "Killer Ig-like receptor-mediated control of natural killer cell alloreactivity in haploidentical hematopoietic stem cell transplantation," *Blood*, vol. 117, no. 3, pp. 764–771, 2011.

[139] H. Fujisaki, H. Kakuda, N. Shimasaki et al., "Expansion of highly cytotoxic human natural killer cells for cancer cell therapy," *Cancer Research*, vol. 69, no. 9, pp. 4010–4017, 2009.

[140] A. Comes, O. Rosso, A. M. Orengo et al., "CD25 regulatory T cell depletion augments immunotherapy of micrometastases by an IL-21-secreting cellular vaccine," *Journal of Immunology*, vol. 176, no. 3, pp. 1750–1758, 2006.

[141] R. Spolski and W. J. Leonard, "IL-21 Is an immune activator that also mediates suppression via IL-10," *Critical Reviews in Immunology*, vol. 30, no. 6, pp. 559–570, 2010.

[142] E. Di Carlo, D. De Totero, T. Piazza, M. Fabbi, and S. Ferrini, "Role of IL-21 in immune-regulation and tumor immunotherapy," *Cancer Immunology, Immunotherapy*, vol. 56, no. 9, pp. 1323–1334, 2007.

[143] D. J. Andorsky and J. M. Timmerman, "Interleukin-21: biology and application to cancer therapy," *Expert Opinion on Biological Therapy*, vol. 8, no. 9, pp. 1295–1307, 2008.

[144] E. Di Carlo, A. Comes, A. M. Orengo et al., "IL-21 induces tumor rejection by specific CTL and IFN-gamma-dependent CXC chemokines in syngeneic mice," *Journal of Immunology*, vol. 172, no. 3, pp. 1540–1547, 2004.

[145] S. I. Ugai, O. Shimozato, L. Yu et al., "Transduction of the IL-21 and IL-23 genes in human pancreatic carcinoma cells produces natural killer cell-dependent and -independent antitumor effects," *Cancer Gene Therapy*, vol. 10, no. 10, pp. 771–778, 2003.

[146] M. Croce, R. Meazza, A. M. Orengo et al., "Immunotherapy of neuroblastoma by an Interleukin-21-secreting cell vaccine involves survivin as antigen," *Cancer Immunology, Immunotherapy*, vol. 57, no. 11, pp. 1625–1634, 2008.

[147] M. Croce, M. V. Corrias, A. M. Orengo et al., "Transient depletion of CD4+ T cells augments IL-21-based immunotherapy of disseminated neuroblastoma in syngeneic mice," *International Journal of Cancer*, vol. 127, no. 5, pp. 1141–1150, 2010.

[148] G. Wang, M. Tschoi, R. Spolski et al., "In vivo antitumor activity of interleukin 21 mediated by natural killer cells," *Cancer Research*, vol. 63, no. 24, pp. 9016–9022, 2003.

[149] T. Kishida, H. Asada, Y. Itokawa et al., "Interleukin (IL)-21 and IL-15 genetic transfer synergistically augments therapeutic antitumor immunity and promotes regression of metastatic lymphoma," *Molecular Therapy*, vol. 8, no. 4, pp. 552–558, 2003.

[150] C. R. Ferrone, M. A. Perales, S. M. Goldberg et al., "Adjuvanticity of plasmid DNA encoding cytokines fused to immunoglobulin Fc domains," *Clinical Cancer Research*, vol. 12, no. 18, pp. 5511–5519, 2006.

[151] I. D. Davis, B. K. Skrumsager, J. Cebon et al., "An open-label, two-arm, phase I trial of recombinant human interleukin-21 in patients with metastatic melanoma," *Clinical Cancer Research*, vol. 13, no. 12, pp. 3630–3636, 2007.

[152] J. A. Thompson, B. D. Curti, B. G. Redman et al., "Phase I study of recombinant interleukin-21 in patients with metastatic melanoma and renal cell carcinoma," *Journal of Clinical Oncology*, vol. 26, no. 12, pp. 2034–2039, 2008.

[153] H. Schmidt, J. Brown, U. Mouritzen et al., "Safety and clinical effect of subcutaneous human interleukin-21 in patients with metastatic melanoma or renal cell carcinoma: a phase I trial," *Clinical Cancer Research*, vol. 16, no. 21, pp. 5312–5319, 2010.

[154] I. D. Davis, B. Brady, R. F. Kefford et al., "Clinical and biological efficacy of recombinant human interleukin-21 in patients with stage TV Malignant melanoma without prior treatment: a phase Lla trial," *Clinical Cancer Research*, vol. 15, no. 6, pp. 2123–2129, 2009.

[155] A. Gowda, A. Ramanunni, C. Cheney et al., "Differential effects of IL-2 and IL-21 on expansion of the CD4+CD25+Foxp3+ T regulatory cells with redundant roles in natural killer cell mediated antibody dependent cellular cytotoxicity in chronic lymphocytic leukemia," *mAbs*, vol. 2, no. 1, pp. 35–41, 2010.

[156] J. Timmerman, C. Byrd, and D. J. Andorsky, "Efficacy and safety of recombinant interleukin-21 and rituximab in relapse/refractory indolent lymphoma," *Journal of Clinical Oncology*, vol. 26, supplement, 2008, abstract 8554.

[157] A. Iannello, M. R. Boulassel, S. Samarani et al., "IL-21 enhances NK cell functions and survival in healthy and

HIV-infected patients with minimal stimulation of viral replication," *Journal of Leukocyte Biology*, vol. 87, no. 5, pp. 857–867, 2010.

[158] N. Strbo, L. De Armas, H. Liu, M. A. Kolber, M. Lichtenheld, and S. Pahwa, "IL-21 augments natural killer effector functions in chronically HIV-infected individuals," *AIDS*, vol. 22, no. 13, pp. 1551–1560, 2008.

[159] A. I. Daud, R. C. DeConti, S. Andrews et al., "Phase I trial of interleukin-12 plasmid electroporation in patients with metastatic melanoma," *Journal of Clinical Oncology*, vol. 26, no. 36, pp. 5896–5903, 2008.

[160] H. N. Lode, R. Xiang, J. C. Becker, S. D. Gillies, and R. A. Reisfeld, "Immunocytokines: a promising approach to cancer immunotherapy," *Pharmacology and Therapeutics*, vol. 80, no. 3, pp. 277–292, 1998.

[161] B. Carnemolla, L. Borsi, E. Balza et al., "Enhancement of the antitumor properties of interleukin-2 by its targeted delivery to the tumor blood vessel extracellular matrix," *Blood*, vol. 99, no. 5, pp. 1659–1665, 2002.

[162] M. Johannsen, G. Spitaleri, G. Curigliano et al., "The tumour-targeting human L19-IL2 immunocytokine: preclinical safety studies, phase I clinical trial in patients with solid tumours and expansion into patients with advanced renal cell carcinoma," *European Journal of Cancer*, vol. 46, no. 16, pp. 2926–2935, 2010.

[163] Z. C. Neal, M. Imboden, A. L. Rakhmilevich et al., "NXS2 murine neuroblastomas express increased levels of MHC class I antigens upon recurrence following NK-dependent immunotherapy," *Cancer Immunology, Immunotherapy*, vol. 53, no. 1, pp. 41–52, 2004.

[164] M. V. Corrias, M. Occhino, M. Croce et al., "Lack of HLA-class I antigens in human neuroblastoma cells: analysis of its relationship to TAP and tapasin expression," *Tissue Antigens*, vol. 57, no. 2, pp. 110–117, 2001.

[165] R. Castriconi, A. Dondero, M. V. Corrias et al., "Natural killer cell-mediated killing of freshly isolated neuroblastoma cells: critical role of DNAX accessory molecule-1-poliovirus receptor interaction," *Cancer Research*, vol. 64, no. 24, pp. 9180–9184, 2004.

[166] D. C. Delgado, J. A. Hank, J. Kolesar et al., "Genotypes of NK cell KIR receptors, their ligands, and Fcγ receptors in the response of neuroblastoma patients to Hu14.18-IL2 immunotherapy," *Cancer Research*, vol. 70, no. 23, pp. 9554–9561, 2010.

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Research Article

WF10 Stimulates NK Cell Cytotoxicity by Increasing LFA-1-Mediated Adhesion to Tumor Cells

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The redox-active chlorite-based drug WF10 (Immunokine) was shown to have modulatory effects on both the innate and adaptive immune system *in vitro* and *in vivo*. Animal studies suggest that WF10 enhances immunity against tumors. One possible explanation for such an effect is that WF10 stimulates natural killer cell cytotoxicity against malignant cells. Here, we show that WF10 regulates human NK cell cytotoxicity in a time-dependent manner, following an S-shaped kinetic with an initial stimulation of activity followed by a decrease in activity relative to the untreated controls. WF10 does not activate NK cells on its own but co-stimulates NK cell activation mediated by different activating receptors. This is mediated by enhancing NK cell adhesion to target cells through promoting the activation of the integrin LFA-1. These data demonstrate a direct effect of WF10 on the cytotoxicity of human NK cells.

1. Introduction

Natural killer (NK) cells are important effector cells for innate immune reactions against viral infections and malignant cells [1]. NK cell effector functions include cellular cytotoxicity and the secretion of cytokines and chemokines. They also fulfill a regulatory role by engaging in cross-talk with diverse cellular components of the immune system [2]. NK cell cytotoxicity is regulated through the recognition of target cells by integrating positive and negative signals from activating and inhibitory receptor-ligand interactions [3]. Many inhibitory NK cell receptors are specific for self-MHC class I and are important to ensure the self-tolerance of NK cells. Loss of MHC class I upon viral infection or malignant transformation can, therefore, result in the so-called "missing-self" reactivity of NK cells. NK cell activation is mediated by a variety of different surface receptors that can recognize specific ligands on transformed or infected cells. Interestingly, freshly isolated resting human NK cells can only be activated by triggering two or more activating receptors in combination [4]. Therefore, all stimulatory receptors for natural cytotoxicity are considered to be coactivating. The only exception seems to be CD16, which is sufficient by itself to induce activation of resting NK cells.

NK cell adhesion to target cells is essential for enabling the interaction between NK cell receptors and their ligands within the immunological synapse and for the directed release of granules towards the attached target cell during NK cell cytotoxicity [5]. NK cell adhesion is mediated by integrins such as LFA-1. The function of LFA-1 is regulated by "inside-out signaling", where signals from other surface receptors influences the affinity of LFA-1 by inducing conformational changes of the molecule, and also affects the avidity of LFA-1 by driving mobility within the cell membrane [6]. This results in clustering of LFA-1 at the site of the NK cell synapse. Adhesion is, therefore, a highly regulated and dynamic process necessary for the function of NK cells.

WF10, or Immunokine, is based on the chlorite ion matrix tetrachlorodecaoxygen and is currently being evaluated in various clinical indications including the adjuvant therapy of inoperable pancreatic cancer and various inflammatory conditions [7–9]. WF10 is known to have various immunological effects by stimulating innate immune functions, while inhibiting adaptive immune functions [10]. In contrast to control animals, sublethally irradiated rats treated with WF10 developed significantly less hematological or solid tumors than controls [11, 12]. This antitumor effect of WF10 could be explained by stimulating the innate natural cytotoxicity against tumors. We, therefore, investigate the effect of WF10 on NK cell cytotoxicity.

2. Materials and Methods

2.1. Materials and Cells. WF10/Immunokine (containing ca. 63 μM chlorite) was provided by Dimethaid GmbH, Wanzleben, Germany. The following antibodies were used: MOPC21 (IgG1 isotype control, Sigma), antiNKp30 (p30-15, generated in our lab), antiNKp46 and antiCD16 (Beckmann Coulter), anti2B4 (Immunotech), antiNKG2D and antiDNAM-1 (R&D Systems). Human ICAM-1-Fc was from R&D Systems, PE-conjugated goat-antihuman Fcγ fragment-specific IgG F(ab′)2 fragment from Jackson Immuno Research. K562, LCL721.221 and P815 cells were maintained as described [13, 14]. Human NK cells were isolated from PBMC by negative selection (Dynal, Invitrogen) and cultured as described [15].

2.2. Cytotoxicity Assay. For ^{51}Cr release assays, target cells were labeled with 100 μCi of ^{51}Cr for 1 h at 37°C. The labeled cells were washed, mixed with NK cells at different effector-to-target (E : T) ratios on a 96-well V-bottom plate and incubated for 4 h at 37°C. For redirected lysis assay NK cells were preincubated with antibodies (0.5 μg/ml final concentration) for 15 min at 37°C before adding the target cells. The supernatant was harvested and ^{51}Cr-release was measured in a γ-counter. Percent specific release was calculated as ((experimental release-spontaneous release)/(maximum release-spontaneous release)) × 100. The ratio between maximum and spontaneous release was at least three in all experiments.

2.3. Real-Time PCR Analysis. NK cells were incubated with or without WF10 for 3 or 18 h. At the end of the incubation cells were lysed in 300 μl MagNA pure lysis buffer containing 1% DTT and mRNA was isolated using the MagnaPure-LC device. Isolated mRNA was transcribed into cDNA using AMV reverse transcriptase (First Strand cDNA synthesis kit (Roche)). Indicated primer sets (Search-LC, Heidelberg) were used with LightCycler-FastStart DNS Sybr Green I Kit (Roche) to amplify the cDNA using the LightCycler according to the manufacture's protocol. The number of transcripts of specific genes in each sample was normalised using the number of transcripts of the house-keeping genes β-actin and cyclophilin b. The transcript number was calculated from a virtual standard curve, obtained by plotting a known input concentration of a plasmid to the PCR cycle number (CP) at which the detected fluorescence intensity reaches a fixed value. For better visualization, a log 2 transformation, of the ratio between WF10-treated and control samples was calculated, as is common for gene expression studies [10].

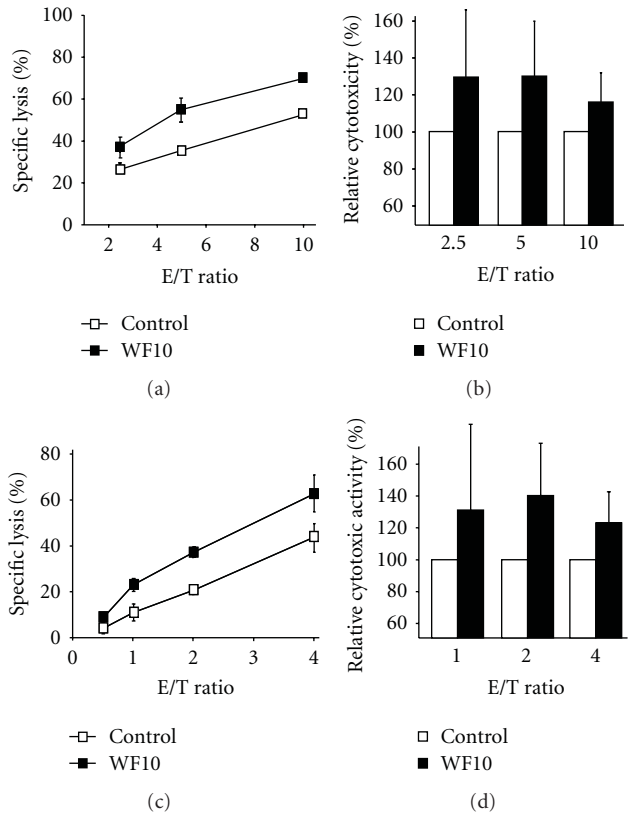

Figure 1: Effect of WF10 on the cytotoxic activity of NK cells. (a, b) Freshly isolated resting human NK cells or (c, d) IL-2 stimulated NK cells were used in a standard 4 h ^{51}Cr-release assays against LCL721.221 target cells with or without the addition of 200 μM WF10 at the indicated effector to target (E/T) ratios. Panels (a) and (c) show the results of representative experiments. In panels (b) and (d) the activity of the WF10-treated cells is shown relative to the cytotoxic activity of control cells (set as 100%) as an average value from (b) 24 or (d) 3 independent experiments. The ratio of each outcome and its control was tested against a value of 1 (equality) by two-sided single-sample t-tests ($^*P < .05$).

2.4. Conjugate Formation Assay and Ligand Complex-Based Adhesion Assay (LC-AA). NK cell-target cell conjugate formation was measured by flow cytometry as described previously [13]. Briefly, freshly isolated NK cells were labeled with the dye PKH67 and LCL721.221 target cells with PKH26 (Sigma). Target and NK cells were combined and incubated with or without WF10 (final concentration of 200 μM chlorite) at 37°C. Reactions were stopped by vortexing, cells were fixed with ice-cold 4% PFA and number of conjugates were determined by FACS analysis. The ligand-complex-based adhesion assay (LC-AA) assesses the activation of the adhesion molecule LFA-1 by FACS analysis of cell bound fluorescently-labeled ICAM-1-complexes and was performed as published [16, 17]. For statistical analysis SPSS Statistics 17.0 was used.

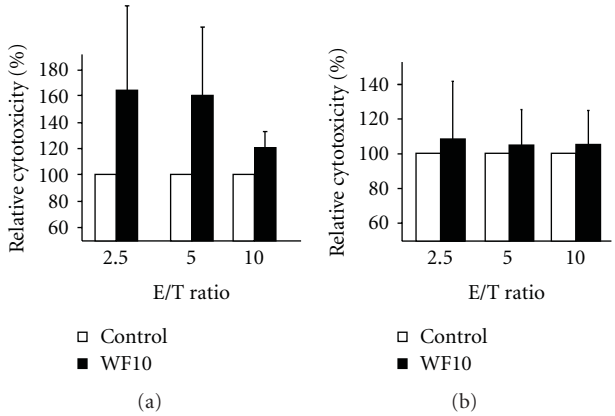

Figure 2: WF10 specifically affects NK cells. (a) Freshly isolated resting human NK cells were preincubated for 5 h with or without 200 μM WF10 in culture medium, washed and then used in a standard 4 h ^{51}Cr-release assays against LCL721.221 target cells at the indicated effector to target (E/T) ratios. (b) LCL721.221 target cells were preincubated with or without 200 μM WF10 in culture medium, washed and then used as targets in a standard 4 h ^{51}Cr-release assays using freshly isolated human NK cells as effectors. The activity of the WF10-treated cells is shown relative to the cytotoxic activity of control cells (set as 100%) as an average value from (a) 4 or (b) 3 independent experiments.

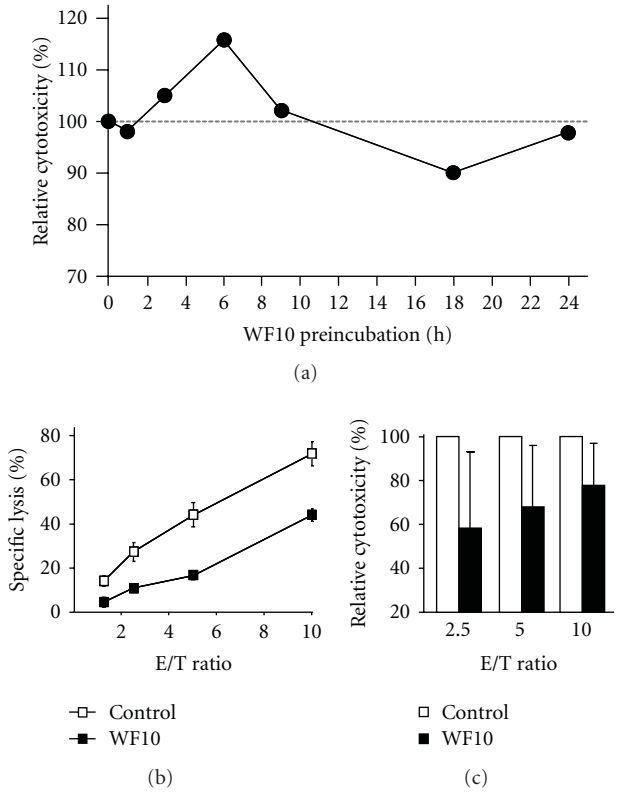

Figure 3: Time-dependent modulation of NK cell responses by WF10. (a) Freshly isolated resting human NK cells or were preincubated for the indicated times with 200 μM WF10 and then used in a standard 4 h ^{51}Cr-release assays against LCL721.221 target cells. Cytotoxic activity was normalized to the untreated control (set as 100%). Shown are the average values of two independent experiments using NK cells from different donors. (b, c) Freshly isolated resting human NK cells were incubated with or without 200 μM WF10 in culture medium for 18 h. Cells were then resuspended in fresh medium and tested as described above. Panel (b) shows the results of a representative experiment. In panel (c) the activity of the WF10-treated cells is shown relative to the cytotoxic activity of control cells (set as 100%) as an average value from 8 independent experiments. The ratio of each outcome and its control was tested against a value of 1 (equality) by two-sided single-sample t-tests ($^*P < .05$).

3. Results and Discussion

3.1. WF10 Can Increase the Cytotoxic Activity of Human NK Cells. To test whether WF10 is able to boost the cytotoxic activity of NK cells, we used freshly isolated human NK cells in a standard 4 h ^{51}Cr-release assay against the MHC class I-negative B cell line LCL721.221. At a therapeutic concentration of 200 μM active chlorite content WF10 significantly enhanced the cytotoxic activity of NK cells (Figures 1(a) and 1(b)). This effect was also seen when PBMC (data not shown) and IL-2-activated human NK cells were used (Figures 1(c) and 1(d)). The enhancement of the NK cell cytotoxicity by WF10 was dose-dependent and could also be observed when NK cells were pretreated with WF10 before the assay (Figure 2(a)). However, WF10 did not directly affect the viability of target cells and pretreatment of target cells with WF10 did not alter their susceptibility to NK-mediated lysis (Figure 2(b)). These data demonstrate that WF10 specifically enhances the cytotoxic activity of NK cells. This effect was not restricted to 721.221 target cells, but also the lysis of the leukemic cell line K562 and the pancreatic cancer cell line Miapaca were enhanced by WF10 (data not shown).

3.2. Time-Dependent Effect of WF10. The increase in NK cell cytotoxicity by WF10 was time-dependent, following an S-shaped kinetic over 24 hours (Figure 3). After an initial boost of activity, WF10 inhibited NK cell cytotoxicity after 18 h of pretreatment (Figures 3(b) and 3(c)). Preincubation of NK cells for 24 hours did no longer result in differences of cytotoxicity between WF10 or control treated cells. The inhibition of NK cell cytotoxicity was not due to a cytotoxic effect of WF10 as we only detected a minor increase in NK cell apoptosis after 18 h of WF10-treatment. However, consistent with previous findings that WF10 affects gene transcription by modulating certain transcription factors in PBMC [10], we detected a reduced expression of cytotoxicity-related genes such as NKG2D, Perforin and DAP12 by quantitative RT-PCR after 18 h of WF10-treatment (Figure 4), which might explain the reduction in cytotoxicity.

3.3. WF10 Enhances NK Cell Cytotoxicity Mediated by Different Activating Receptors. Next we wanted to test if the WF10-mediated enhancement of NK cell activity was mediated through a specific activating receptor. Therefore, we triggered NK cell cytotoxicity through NKp30, NKG2D or 2B4 in a redirected lysis assay. In line with the concept of NK cell coactivation [4], triggering of any of these

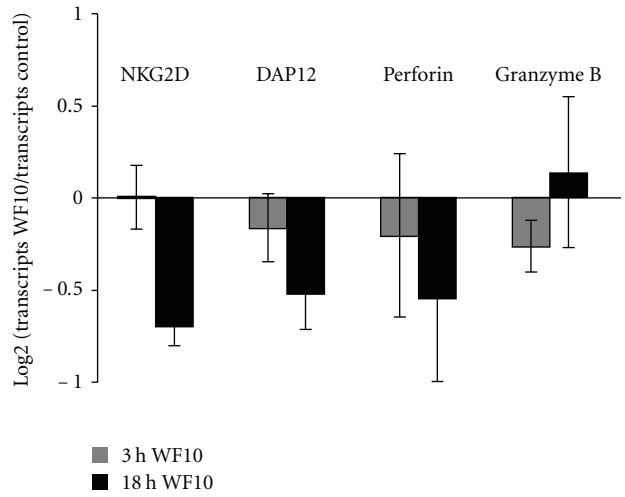

Figure 4: The effect of WF10 on the transcription of NK cell genes. Freshly isolated NK cells were incubated with WF10 (at a chlorite concentration of 200 μM) for 0, 3, or 18 h and mRNA was isolated. The indicated transcripts were quantified by quantitative RT-PCR. The number of transcripts of individual genes in each sample was normalised using the number of transcripts of the house-keeping genes β-actin and cyclophilin b. The number of transcripts in the WF10-treated sample was compared with the number of transcripts in the control sample using the formula log 2 (transcripts WF10 sample/transcripts control sample). Average values and standard deviation from 5 independent experiments with different blood donors are shown.

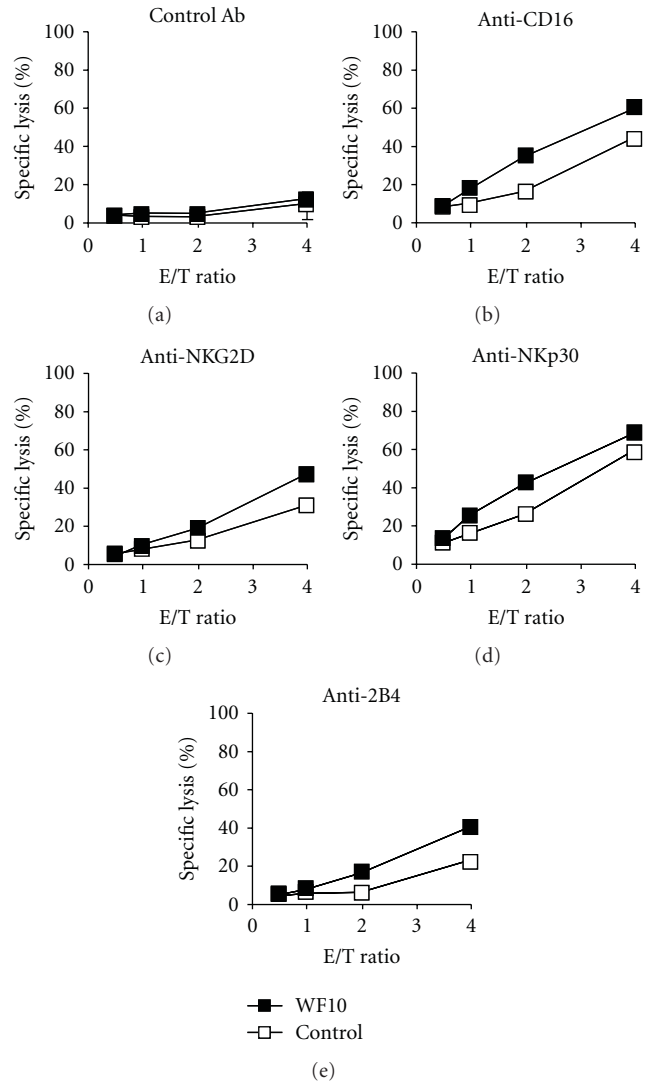

Figure 5: Enhancement of receptor-mediated cytotoxicity of IL-2-activated NK cells by WF10. IL-2-activated NK cells were tested in a redirected-lysis assay against murine P815 cells. NK cells were stimulated using a (a) control antibody, or by antibodies directed against the activating receptors (b) CD16, (c) NKG2D, (d) NKp30, or (e) 2B4. Where indicated, WF10 was added to the assay medium at a final chlorite concentration of 200 μM. Average values of triplicates are shown with the standard deviation. One representative of three independent experiments with different donors is shown.

receptors on freshly isolated NK cells did not stimulate cytotoxicity. Also the addition of WF10 did not change this. However, when we used IL-2-activated NK cells, WF10 could enhance NK cell cytotoxicity mediated by CD16, NKG2D, NKp30 and 2B4 (Figure 5). This suggests that WF10 acts independently of one specific receptor proximal signaling pathway. Interestingly, WF10 can only costimulate NK cell cytotoxicity but does not lead to NK cell activation on its own.

3.4. WF10 Enhances Target Cell Adhesion and Co-Stimulates the Activation of LFA-1. As WF10 acted independently of specific receptor signaling pathways, we tested its effect on NK cell adhesion to target cells. This adhesion is essential for NK cell cytotoxicity. It allows the interaction of NK cell receptors with their ligands on the target cells and is essential for the directed release of cytotoxic granules. During the first 90 minutes of conjugate formation between freshly isolated NK cells and 721.221 target cells WF10 showed only a minor effect. However, at later time points WF10-treatment resulted in an increase in the amount of conjugates (Figure 6(a)). LFA-1 is an important adhesion molecule on NK cells. The affinity and avidity of LFA-1 for its ligand ICAM-1 is regulated by inside-out-signals that can derive from cytokines or the activation of cytotoxicity receptors [6]. We, therefore, tested the binding activity of LFA-1 using a ligand-complex-based adhesion assay (LC-AA) [16]. In this assay fluorescently-labeled ICAM-1 complexes are used for the staining of cells in a FACS-based analysis. These complexes will only bind to LFA-1 in its high affinity or high avidity conformation. Freshly isolated NK cells did not show binding of ICAM-1 (Figures 6(b) and 6(c)), indicating that LFA-1 is not activated in resting NK cells. The addition of WF10 did not change this, which confirms that WF10 cannot stimulate NK cell activation by its own, but can only costimulate other activation signals. To induce inside-out signaling we stimulated the NK cells with IL-15, which resulted in LFA-1 activation (Figure 6(b)). WF10 significantly increased the LFA-1 activation after IL-15 incubation (Figures 6(b) and 6(c)). These data suggest that WF10 can enhance NK cell

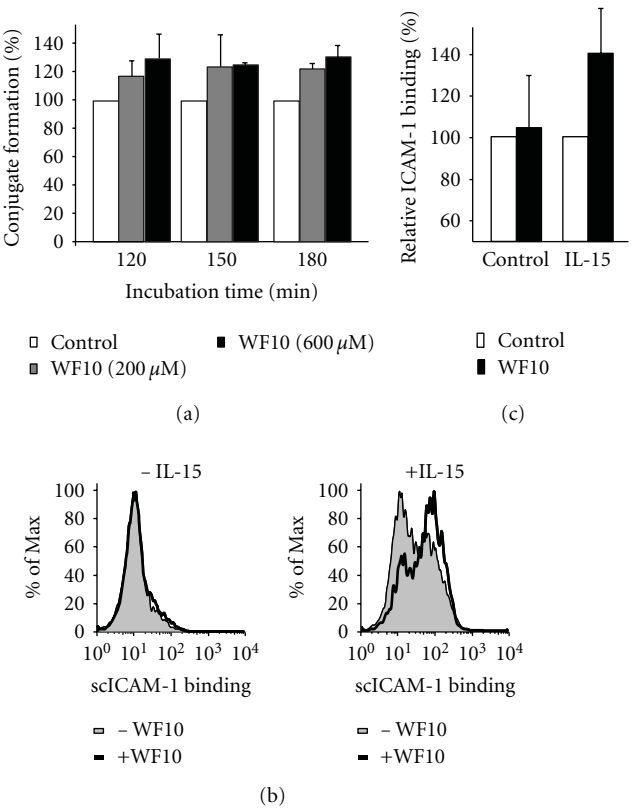

Figure 6: WF10 enhances NK cell adhesion. (a) NK cells and 721.221 cells were labeled with a different fluorescent dye, washed, and then incubated together with or without WF10 (200 or 600 μM). At the indicated time points the mixed suspension was fixed with ice-cold 4% PFA and analyzed by FACS. The amount of NK: 721.221 conjugates formed by the cells without WF10 was used as a reference (100%). Average values and the standard deviation from 3 independent experiments with different blood donors are shown. (b, c) Freshly isolated NK cells were incubated with PE-conjugated ICAM-1 complex (scICAM-1) with or without WF10 for 3 h. Samples were either left unstimulated (b, left panel), or treated with IL-15 (100 U/ml) (b, right panel). The amount of bound ICAM-1 complexes was measured by FACS. A representative experiment is shown in (b). The ICAM-1 binding to control cells (MFI 44.2 ± 20.4) and IL-15 stimulated cells (MFI 126 ± 60.3) was used as a reference (100%). (c) Average values and standard deviations from 5 independent experiments with different blood donors are shown. The ratio of each outcome and its control was tested against a value of 1 (equality) by two-sided single-sample t-tests ($^*P < .05$).

adhesion to target cells by costimulating inside-out signaling resulting in higher LFA-1 activity. This would explain the higher cytotoxic activity of NK cells after WF10-treatment and suggests that rather than hyperactivating a few NK cells to kill more frequently, WF10 may increase the number of NK cells involved in cytotoxicity at a given time, so that cells that were previously insufficiently activated are now recruited to the killing process by WF10. This would be in line with our finding that WF10 can costimulate NK cells, but not activate them on its own.

4. Concluding Remarks

WF10 was shown to inhibit the development and promotion of leukemia in animal models [11, 12]. The role of oxidative species in cancer is complex and has been extensively studied in the last decades. ROS appear to have a dual role in tumor development and growth. Oxidative species contribute to DNA damage, a major step in carcinogenesis and progression, but they can also act in pro-apoptotic signaling pathways and, therefore, have antitumor effects [18]. Due to this dual role, some anticancer strategies are based on pro-oxidant mechanisms and some on antioxidant mechanisms [19]. In addition to these direct effects, we have shown in this study that WF10 can stimulate NK cell cytotoxicity through promoting LFA-1-mediated adhesion to tumor cells. As NK cells are known to be important effector cells against hematological malignancies [20], it may be promising to combine WF10 with other strategies of NK cell-based immunotherapy against cancer. However, the effects of WF10 on immune function appears to be modulatory, rather than either stimulatory or inhibitory. It is, therefore, expected that a treatment with WF10 could only temporary enhance NK cell functions, followed by a phase of reduced NK cell activity. It is unclear if this transient inhibition of NK cell cytotoxicity, possibly mediated by the down-regulation of cytotoxicity-related genes such as NKG2D, DAP12, or perforin, would lead to a gap in immune defense mechanisms. However, this transient inhibition of NK cell cytotoxicity may be overcome by combining the WF10-based therapy with stimulatory substances, such as Interleukin-15, for which a synergistic effect in combination with WF10 has been shown in our study. Additionally, our data would suggest that it may be beneficial to administer WF10 in individual boosts rather than maintaining a constant drug level in order to exploit its influence on NK cell activity.

Author Contribution

L. Kühne performed research; M. Konstandin and Y. Samstag helped with LC-AA binding assay; L. Kühne, S. Meuer, T. Giese and C. Watzl designed research and analyzed the data; C. Watzl and L. Kühne wrote the manuscript.

Abbreviations

LC-AA: Ligand-complex-based adhesion assay,
LFA-1: Lymphocyte function-associated antigen-1,
NK: Natural killer,
PBMC: peripheral blood mononuclear cells.

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References

[1] E. Vivier, E. Tomasello, M. Baratin, T. Walzer, and S. Ugolini, "Functions of natural killer cells," *Nature Immunology*, vol. 9, no. 5, pp. 503–510, 2008.

[2] A. Moretta, "Natural killer cells and dendritic cells: rendezvous in abused tissues," *Nature Reviews Immunology*, vol. 2, no. 12, pp. 957–964, 2002.

[3] L. L. Lanier, "Up on the tightrope: natural killer cell activation and inhibition," *Nature Immunology*, vol. 9, no. 5, pp. 495–502, 2008.

[4] Y. T. Bryceson, M. E. March, H. G. Ljunggren, and E. O. Long, "Synergy among receptors on resting NK cells for the activation of natural cytotoxicity and cytokine secretion," *Blood*, vol. 107, no. 1, pp. 159–166, 2006.

[5] J. C. Stinchcombe and G. M. Griffiths, "Secretory mechanisms in cell-mediated cytotoxicity," *Annual Review of Cell and Developmental Biology*, vol. 23, pp. 495–517, 2007.

[6] B. H. Luo, C. V. Carman, and T. A. Springer, "Structural basis of integrin regulation and signaling," *Annual Review of Immunology*, vol. 25, pp. 619–647, 2007.

[7] I. A. Malik, I. Moid, S. Haq, and M. Sabih, "A double-blind, placebo-controlled, randomized trial to evaluate the role of tetrachlorodecaoxide in the management of chemotherapy-induced oral mucositis," *Journal of Pain and Symptom Management*, vol. 14, no. 2, pp. 82–87, 1997.

[8] V. Veerasarn, W. Boonnuch, and C. Kakanaporn, "A phase II study to evaluate WF10 in patients with late hemorrhagic radiation cystitis and proctitis," *Gynecologic Oncology*, vol. 100, no. 1, pp. 179–184, 2006.

[9] S. Penpattanagul, "Reduced incidence and severity of acute radiation mucositis by WF10 (IMMUNOKINE) as adjunct to standard of cure in the management of head & neck cancer patients," *Journal of the Medical Association of Thailand*, vol. 90, no. 8, pp. 1590–1600, 2007.

[10] T. Giese, M. S. McGrath, S. Stumm, H. Schempp, E. Elstner, and S. C. Meuer, "Differential effects on innate versus adaptive immune responses by WF10," *Cellular Immunology*, vol. 229, no. 2, pp. 149–158, 2004.

[11] S. R. Kempf, K. Blaszkiewitz, R. E. Port, and S. Ivankovic, "Influence of tetrachlorodecaoxide (Ryoxon) on the development of leukemia after total-body gamma-irradiation," *Oncology*, vol. 51, no. 6, pp. 510–514, 1994.

[12] S. R. Kempf, R. E. Port, and S. Ivankovic, "Anticarcinogenic effect of tetrachlorodecaoxide after total-body gamma irradiation in rats," *Radiation Research*, vol. 139, no. 2, pp. 226–231, 1994.

[13] P. C. Raemer, K. Kohl, and C. Watzl, "Statins inhibit NK-cell cytotoxicity by interfering with LFA-1-mediated conjugate formation," *European Journal of Immunology*, vol. 39, no. 6, pp. 1456–1465, 2009.

[14] R. Bhat and C. Watzl, "Serial killing of tumor cells by human natural killer cells—enhancement by therapeutic antibodies," *PLoS ONE*, vol. 2, no. 3, article e326, 2007.

[15] P. Eissmann and C. Watzl, "Molecular analysis of NTB-A signaling: a role for EAT-2 in NTB-A-mediated activation of human NK cells," *Journal of Immunology*, vol. 177, no. 5, pp. 3170–3177, 2006.

[16] M. H. Konstandin, U. Sester, M. Klemke, T. Weschenfelder, G. H. Wabnitz, and Y. Samstag, "A novel flow-cytometry-based assay for quantification of affinity and avidity changes of integrins," *Journal of Immunological Methods*, vol. 310, no. 1-2, pp. 67–77, 2006.

[17] S. C. Hoffmann, A. Cohnen, T. Ludwig, and C. Watzl, "2B4 engagement mediates rapid LFA-1 and actin-dependent NK cell adhesion to tumor cells as measured by single cell force spectroscopy," *Journal of Immunology*, vol. 186, no. 5, pp. 2757–2764, 2011.

[18] M. Valko, C. J. Rhodes, J. Moncol, M. Izakovic, and M. Mazur, "Free radicals, metals and antioxidants in oxidative stress-induced cancer," *Chemico-Biological Interactions*, vol. 160, no. 1, pp. 1–40, 2006.

[19] J. Wang and J. Yi, "Cancer cell killing via ROS: to increase or decrease, that is a question," *Cancer Biology and Therapy*, vol. 7, no. 12, pp. 1875–1884, 2008.

[20] M. Terme, E. Ullrich, N. F. Delahaye, N. Chaput, and L. Zitvogel, "Natural killer cell-directed therapies: moving from unexpected results to successful strategies," *Nature Immunology*, vol. 9, no. 5, pp. 486–494, 2008.

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Review Article

How the Virus Outsmarts the Host: Function and Structure of Cytomegalovirus MHC-I-Like Molecules in the Evasion of Natural Killer Cell Surveillance

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Natural killer (NK) cells provide an initial host immune response to infection by many viral pathogens. Consequently, the viruses have evolved mechanisms to attenuate the host response, leading to improved viral fitness. One mechanism employed by members of the β-herpesvirus family, which includes the cytomegaloviruses, is to modulate the expression of cell surface ligands recognized by NK cell activation molecules. A novel set of cytomegalovirus (CMV) genes, exemplified by the mouse m145 family, encode molecules that have structural and functional features similar to those of host major histocompatibility-encoded (MHC) class I molecules, some of which are known to contribute to immune evasion. In this review, we explore the function, structure, and evolution of MHC-I-like molecules of the CMVs and speculate on the dynamic development of novel immunoevasive functions based on the MHC-I protein fold.

1. Introduction

Mammals are susceptible to a wide range of infectious agents, including, but not limited to, viruses, bacteria, and protozoan parasites. While many microbes cause debilitating illnesses, are responsible for much morbidity and mortality worldwide, and garner much of the public's attention, other organisms stealthily invade their hosts, establish lifelong infection, and remarkably, cause little or no symptoms in healthy individuals. CMVs are examples of microbes that establish asymptomatic, latent, and lifelong infections, revealing themselves only when the host's immune system is compromised. Virus survival in the face of an intact immune system is accomplished through subversion of antiviral immunity by an arsenal of virally encoded proteins, termed immunoevasins, that specifically target key molecular recognition steps necessary for an immune response. The interplay of evolutionary diversification of immunoevasins with the defense mechanisms of the host results in a dynamic balance permitting the survival of both the host and the infectious organism. Among the many viral infections of fundamental interest that have been well studied are the species-specific large DNA viruses of the β-herpesvirus family, of which the CMVs are representative members [1, 2]. The human CMV (HCMV) as well as its murine relative (MCMV) [3] and other species such as the guinea pig (GPCMV) [4], rhesus (RhCMV) [5–9], and chimpanzee (CCMV) [10, 11] have been the subject of recent studies designed to understand not only the basic genetics, biochemistry, and biology of these complex organisms but also to discern the immune responses of their hosts, with an ultimate goal of developing effective vaccines to alleviate pathogenic effects of the viruses. MCMV infection is a model for HCMV infection in humans, because of similarities in viral life cycle, genome structure, and host immune response [12–15]. These viruses exhibit similarities in the life cycle of acute infection, persistence and

latent infection or superinfection, and reactivation under conditions of immune suppression [8, 16]. The subject of this review is a structural, genetic, and functional analysis of a set of genes and their encoded glycoproteins that have been adapted by MCMV to assure the continued survival of the virus. Because of the structural similarities of these encoded proteins to MHC-I and other MHC-I-like molecules of the host, we argue that these molecules were derived from lateral (horizontal) genetic transmission from host to virus.

HCMV is a serious and opportunistic pathogen that affects 45–100% of the adult population. Seroprevalence is influenced by age, race and ethnicity, sex, and socioeconomic status where frequency of infection is highest in urban areas [17]. Primary infections are usually asymptomatic in healthy individuals but can cause significant morbidity in immunocompromised patients such as those with AIDS or cancer, and in individuals undergoing therapeutic immunosuppression in the course of solid organ transplantation. Congenital infection resulting from primary maternal infection that has a rate of 1–4% is also a major concern, leading to long-term sequelae such as neurodevelopmental disabilities, including mental retardation and sensorineural hearing loss [18].

2. Background

2.1. The Immune Response to CMV Infection. Mammalian cells possess sophisticated mechanisms that telegraph their health status to the cell surface for recognition by inflammatory and immune cells. The vertebrate host responds to CMV infection using the full battery of specialized cells of the immune system: NK-cells, B cells, and T cells of both cytolytic ($CD8^+$) and helper ($CD4^+$) lineages. Aspects of both acute and chronic CMV disease may be controlled by antibodies, NK, and other cells of the innate immune system, as well as by $CD8^+$ and $CD4^+$ T cells. Such cells of the immune system can either directly kill the virus-infected cells or produce bioactive molecules that exert direct and indirect effects on the innate and adaptive arms of the immune response. Two main cellular mechanisms alert the immune system to an infected or stressed state: NK-cell and T-cell recognition and activation.

During viral infection, NK-cells offer an important first line of defense that limits viral expansion at a time when specific immunity has not yet fully developed. But the virus has evolved countermeasures to balance this formidable NK surveillance [19] (see Figure 1). Following the initial NK response, the host develops adaptive $CD8^+$ and $CD4^+$ T cell responses [20–22].

2.2. Viral Evasins. Viruses have two major life cycle advantages that allow them to counter the host's immune response: their rapid generation time permits them to accumulate genetic variants that allow them to subvert the immune response, and viruses with large genomes have the capacity to devote extensive amounts of genetic material to functions that may provide even slight evolutionary advantage. As a group, the CMV have genomes that are colinear as in the case of MCMV and HCMV, that may be as large as

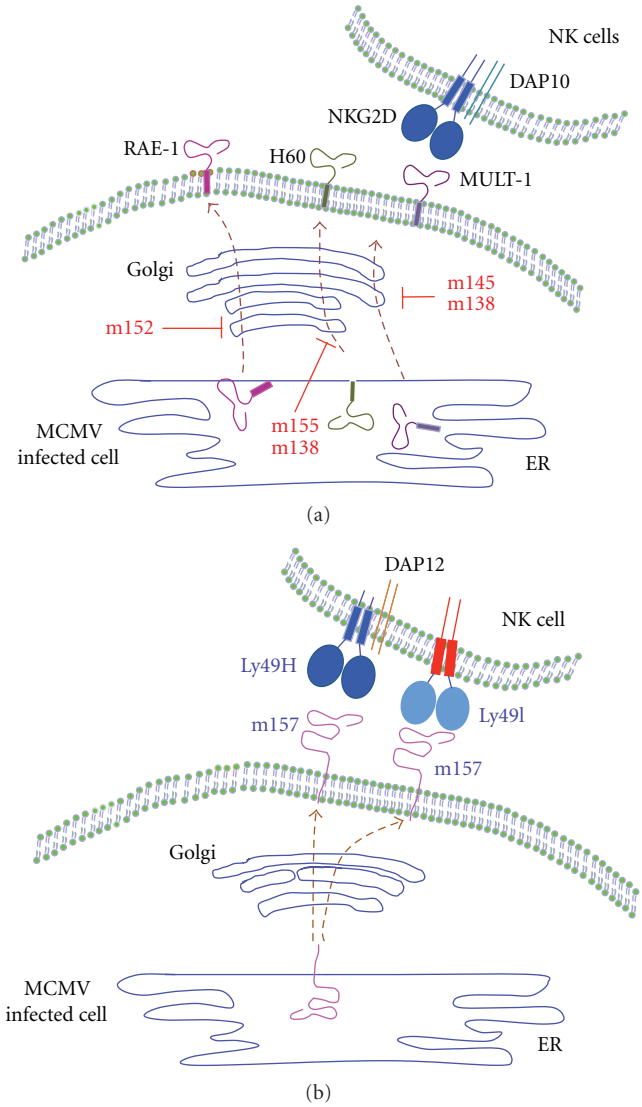

Figure 1: MCMV-encoded proteins disrupt NK-cell recognition of infected cells. During MCMV infection, the surface expression of the stress-induced molecules, ligands for NKG2D, is downregulated: m152 downregulates all isoforms of RAE-1, m145 and m155 interfere with H60 and MULT-1, respectively, and m138 downregulates H60, MULT-1, and RAE-1ε (a). m157 binds both inhibitory NK receptor, Ly49I, and activating NK receptor, Ly49H (b).

230 kb, and that encode as many as 170 open reading frames (ORFs), of which about one-third is required for essential viral functions. About half of the identified genes in MCMV have HCMV homologues [23, 24]. Although the genetic and functional analysis of all of these genes has not yet been performed, studies of many of them indicate a role in curtailing NK-cell recognition of the virus-infected cell or in interfering with antigen processing and presentation to $CD8^+$ T cells. Of particular interest to our studies of MHC-I-like molecules of the virus is the m145 family of genes (m17, m145 to m158), several of which have been shown to contribute to viral fitness. (Originally denoted the m145 family, current BLAST [25] searches of the protein database identify their

encoded proteins as members of the "m157 superfamily.") Remarkably, most of these genes map to the extreme right end of the MCMV genome while the more highly conserved essential functions of the virus map to the center. Also, another set of genes, some of which play a similar role immunoevasion, map to the extreme left of the MCMV genome. These are known as the m02 family (genes m02 to m16) and some evidence suggests that they can impair T-cell receptor-mediated recognition of MHC-I/peptide complexes that lead to $CD8^+$ T-cell activation [26, 27].

2.3. NK Receptors in Viral Infection. During the early stages of MCMV infection, the host immune response is dominated by NK-cell activation and the resulting cytolysis of virus-infected cells. The activation of NK-cells is regulated by a balance of signals delivered through activating or inhibitory receptors. These surface molecules either bind classical MHC-I molecules or MHC-I homologues and are classified into two families: C-type lectin-like (Ly49, NKG2D and CD94/NKG2) and immunoglobulin-like (KIRs and LIRs) as reviewed elsewhere [28–30].

2.4. NKG2D. The infected cell initiates a complex stress response, leading to increased cell surface production of a spectrum of molecules including MICA or MICB, and members of the ULBP family in the human [31–34], or RAE-1 ($\alpha, \beta, \gamma, \delta, \varepsilon$), MULT-1, and H60 in the mouse [35–39]. These MHC-I-like stress-induced cell surface molecules are ligands for the NK-cell activation receptor, NKG2D, the best characterized NK activating receptor. NKG2D lacks a signaling motif of its own, and thus requires association with either the DAP10 or DAP12 adapter molecules [40]. In the mouse, NKG2D Short pairs with either DAP10 or DAP12 [41, 42], while NKG2D Long interacts exclusively with DAP10. Human NKG2D, by contrast, only has the L isoform and thus interacts exclusively with DAP10 [43]. The direct interaction of NKG2D with any of the NKG2D ligands activates the NK-cell and initiates its cytokine and cytolytic program, resulting in the killing of the virus-infected cell.

To counter host NK surveillance, the virus has evolved strategies to attenuate the host cell expression of the NKG2D ligands, which it accomplishes through the expression of some m145 family members early in infection. In particular, the m152, m145, and m155 glycoproteins, as well as the unrelated m138, each downregulates one or more NKG2D ligands. m152, encoding the gp40 glycoprotein, not only controls the surface expression of classical MHC-I, but also downregulates surface expression of RAE-1 molecules. Although this regulatory function of m152 has been recognized for several years [44, 45], evidence for direct interaction of m152 with RAE-1 has only been demonstrated recently. Studies show binding of m152 with RAE-1 isoforms β, γ, and δ and establish a relationship between the effectiveness of RAE-1 attenuation with the intrinsic affinity of the m152/RAE-1 interaction [46]. In a manner similar to that of the m152/RAE-1 interaction, the m145-encoded glycoprotein downmodulates the expression of MULT-1 [47], and m155 blocks H60 surface expression [48]. m138, originally considered a viral Fc receptor, also regulates both MULT-1 and H60 as well as RAE-1ε. In addition, it also affects B7-1 (CD80) expression on dendritic cells (DCs) which impairs DC stimulation of CTLs [49]. The functions of these MCMV genes have been established in part by the judicious exploitation of deletion viruses such as the Δm152 mutant, that clearly fails to downregulate both MHC-I and RAE-1 [45, 50], the Δm138 mutant that is deficient in H60 and MULT-1 regulation, and the Δm155 virus that attenuates the NK response *in vivo* and partially restores H60 expression on virus-infected cells [48].

The intriguing structural question raised by the paired interactions of members of the m145 family with NKG2D ligands is how precisely do these viral MHC-I-like molecules function. The high-resolution X-ray crystallographic structures of several of these viral MHC-I-like molecules are now known. In addition, the structures of some of the NKG2D ligands have been determined. These structures offer further insight not only into the function of the viral MHC-I-like molecules, but also into their evolution.

2.5. Ly49 Receptors. Major advances in our understanding of the role of NK receptors in the immune response to viral infection derived from studies of the Ly49 family in the mouse and of the KIR family in the human. These are cell surface receptors, expressed primarily on NK-cells, that interact either with host classical MHC-I molecules, or, in several notable examples, with virus-encoded ligands. The Ly49 family members are either inhibitory (such as Ly49A, Ly49C, or Ly49I), or activating (such as Ly49H or Ly49P). Similar functions are contributed by the KIRDL inhibitory receptors and the KIRDS activating receptors in the human, but our discussion will be confined to the mouse molecules.

The inhibitory receptors, with Ly49A serving as the prototype, recognize classical MHC-I on host cells, and thus deliver a tonic inhibitory signal to the NK-cell, through their cytoplasmic immunoreceptor tyrosine-based inhibition motifs (ITIMs). With decreased MHC-I expression on the virus-infected cell, the strength of the inhibitory signal decreases, and concurrent activating signals dominate, leading to lysis of the virus-infected cell. Such a mechanism, the basis of the missing self-hypothesis [51] has been well-characterized for the interaction of Ly49A with its MHC-I ligand $H\text{-}2D^d$ [52, 53]. The importance of interactions of Ly49A and other inhibitory receptors with their MHC-I ligands in NK-cell education or licensing has also recently been explored [54, 55].

Some activating receptors such as Ly49H, in contrast to NKG2D, which exploits stress-induced ligands, do not have known self-MHC-I ligands, but instead interact strongly with some CMV-encoded molecules. Ly49H is expressed in MCMV-resistant mouse strains and binds a viral member of the m145 family, m157, which is expressed at the cell surface as a glycophosphatidylinositol (GPI)-linked glycoprotein early in infection. Ly49H deficient mice are MCMV sensitive, and transgenic expression of Ly49H confirms that this activating receptor alone can account for viral resistance. In mouse strains susceptible to MCMV infection such as

129/J, there is no Ly49H gene, but rather one encoding an inhibitory receptor Ly49I^{129}, that interacts strongly with m157 [56, 57]. Thus, it would appear that m157 evolved initially in the setting of hosts that expressed Ly49I-like activities, resulting in improved viral survival. As the virus became more virulent, mouse evolution settled on the solution of shuffling the Ly49I-binding activity (residing in its extracellular domain) onto the signaling module of an activating receptor and thus became Ly49H, conferring resistance to viruses that express m157 [58]. In experiments designed to examine the evolution of virus resistance to host NK activity, it was shown that when MCMV is passaged repeatedly through resistant Ly49H$^+$ mice, m157 mutations accumulate rapidly, permitting the virus to escape the NK immunosurveillance due to Ly49H [59]. Recent studies of a variety of naturally occurring m157 variants indicate that many are incapable of binding Ly49H (from C57BL/6), but can interact with Ly49C inhibitory receptors from several different strains [60]. Thus, the effects of the differential interactions of Ly49 activating and inhibitory NK receptors on the evolution of viral MHC-like ligands, such as m157, may prove to be even more complex than previously thought.

There are some mouse strains that lack Ly49H but are resistant to MCMV infection through other NK-cell-mediated mechanisms. An example is the Ma/My mouse whose resistance is genetically dependent on the presence of genes encoding an activation receptor Ly49P, and H-2D^k [61]. Epistatic interactions of these genes (or their gene products) confer resistance to MCMV. The Ly49P dependent activation of NK-cells is blocked by an antibody to H-2D^k [61, 62]. In addition to H-2D^k and Ly49P, the viral resistance of Ma/My also requires m04, a gene encoding gp34, a glycoprotein that escorts MHC-I to the surface and that inhibits recognition by CD8$^+$ CTL. A Δm04 mutant of MCMV abrogates the resistance of Ma/My mice [30, 62, 63]. The mechanism by which these three gene products, Ly49P and H-2D^k of the host, and m04 of MCMV, cooperatively generate viral resistance remains unclear.

3. Biochemistry, Structure, and Evolution of Viral MHC-I-Like Molecules

3.1. Interaction of MHC-I-Like MCMV Molecules and NK Receptors. Studies of the function of the MHC-I-like genes of the CMVs have largely relied on experiments with mutant viruses with engineered deletions of the relevant genes, on detection of cell surface expression of host proteins following infection or transfection, or on immunoprecipitation (pull-down) experiments using specific antibodies. Although such experiments support the conclusions that some of these viral MHC-I-like molecules either downregulate or impair the recognition of particular ligands, they fail to explain the precise molecular mechanism(s) involved in such regulatory effects [39, 44, 50]. To this end, several laboratories have directed efforts to engineer recombinant forms of the viral MHC-I-like proteins and their ligands and to measure these interactions in well-defined *in vitro* systems. Specifically, the interactions of MCMV m152 [46]and m157 [64, 65]and of HCMV UL18 [65] have been examined in this way.

The engineering, expression, and purification of soluble forms of the extracellular domains of m152 and RAE-1β, -1γ (expressed in BALB/c), and RAE-1δ (C57BL/6) generated the reagents for size exclusion binding assays, analytical ultracentrifugation (AUC), and isothermal titration calorimetry (ITC), based on the hypothesis that the ectodomains m152 and RAE-1 isoforms interact directly. Recombinant m152, prepared in insect cells, interacted well with RAE-1 molecules refolded from *E. coli* inclusion bodies. Affinities for the interactions were measured by AUC with K_ds of RAE-1γ (1 μM) > RAE-β(3 μM) > RAE-1δ (30 μM) [46], which may be compared with the K_d of the interaction of murine NKG2D with several RAE-1 isoforms (340–730 nM) [66]. The hierarchy of affinities of the different isoforms paralleled the effectiveness in the downregulation of RAE-1 by m152. In addition, these studies confirmed the predicted 1 : 1 stoichiometry of the m152 : RAE-1 interaction.

The interaction between m157 and Ly49 NK receptors was first detected using m157-fusion proteins [56], or using an Ly49H-reporter cell and an m157 transfectant [67]. In experiments employing recombinant m157, Ly49H, and Ly49I and surface plasmon resonance (SPR) as well as ITC, the affinity of Ly49I for m157 was determined to have a K_d of 0.2 μM with a 1 : 1 stoichiometry, a stronger affinity than Ly49's interaction with standard MHC-I ligands (1–80 μM) [64, 68, 69].

UL18, an HCMV molecule that interacts with LIR1 (also known as ILT2 or CD85j), an inhibitory receptor expressed widely on monocytes, DCs, B cells, and some T cells and NK-cells, has also been studied quantitatively by SPR methods. The interaction of LIR1 with UL18 ($K_d \sim 10^{-2}\,\mu$M) is >1000-fold stronger than that of LIR1 with classical MHC-I [65]. It is interesting to note that the physical interaction between UL18 and the human NK-cell activating receptor, NKG2C/CD94, has been estimated to have a K_d of about 10 to 100 μM [70].

The quantitative measure of direct binding interactions between viral MHC-I like proteins and their ligands reflects the strength with which these evasins can compete with host protective or inhibitory mechanisms. Knowledge of the structural details of these interactions contributes to our understanding of the evolution and molecular mechanism of such viral MHC-I mimics.

3.2. Structural Characteristics of CMV MHC-I-Like Molecules

3.2.1. Amino Acid Sequence Similarities. The first CMV gene identified as an MHC-I homolog was H301 (now known as UL18) of HCMV, which was shown to encode a protein with 20% similarity to classical MHC-I proteins [71]. Subsequently, with the complete DNA sequence determination of the MCMV genome and bioinformatic analysis of its ORFs [23], m144 was shown to have amino acid sequence similarity to classical MHC-I proteins. Reexamination of ORFs of MCMV using more recently developed computational tools suggested the existence of other genes that encode MHC-I-like molecules [72]. Simple alignment of classical MHC-I molecules from human and mouse reveals obvious sequence

similarity over 267 amino acid residues of the extracellular domain with scores of 81% similarity and 71% identity (see Figure 2(a)). When UL18 and m144 are included in the sequence alignment, similarities, particularly in the conservation of cysteine residues, are still evident, although UL18 is only 24% identical with HLA-A2, and m144 is about 19% identical to H-2D^d (Figure 2(b)). However, efforts to align all the members of the m145 family from MCMV reveal profound differences in sequence and considerable problems in selecting appropriate computational parameters for the best alignment (Figure 2(c)). Sequence identity scores for the m145 family as compared with the classical MHC-I molecule H-2D^d range from 6.2 (for m151) to 24.4% (for m144).

These rather marginal sequence similarities and the inherent ambiguities in evaluating the alignments of cysteine residues demand a more objective three-dimensional structural comparison.

3.2.2. Three-Dimensional Structures of Viral Evasins. The structures of three members of the m145 family (m144 [73], m153 [74], and m157 [64]) have been solved, as well as those of the HCMV UL18 [75, 76]. In addition, a putative evasin of the tanapox virus 2L, which, remarkably, is also an MHC-I-like molecule [77], has been examined in structural detail. Furthermore, structures of several other HCMV molecules, US2 and UL16, that function as immunoevasins, but are structurally related to the immunoglobulin superfamily and not related to the MHC-I family, have also been determined [78, 79].

Early studies of m144 suggested that it inhibited the recognition of virus-infected cells by NK-cells *in vivo* [80], and that m144 expression in tumor cells conferred resistance to NK-cell killing [81]. However, these results are controversial and there remains no consensus as to the function of the m144 glycoprotein.

Our lab has examined the expression and structure of m144 [73](PDB [82] code 1U58) (see Figure 3, Table 1). Biochemical analysis [83] of m144 revealed the lack of co-purifying bound peptides, a result confirmed by transfection studies [73] that revealed the lack of a requirement for bound peptide for cell surface expression. The X-ray structure [73] shows a typical MHC-I fold consisting of α1 and α2 helices supported by a floor of antiparallel β strands and connected via a loop to an immunoglobulin-like α3 domain. Although m144 cocrystalized with β2-microglobulin (β2m), located in a canonical position beneath the β strand floor, expression studies [42, 84] showed that there is no absolute β2m requirement for folding and cell surface display. The $\alpha1\alpha2$ domain unit is stabilized by two disulfide bonds: one that is similar in orientation to that found in classical MHC-I molecules (joining the β5 strand to the α2 helix) and another unique one that links the α1 helix to β4. The disulfide in the immunoglobulin-like α3 domain is conserved. The structure reveals truncated α1 and α2 helices, a narrowed groove, and a modified β2m interface. An unstructured stretch of 13 amino acids not seen in the electron density map may be indicative of a flexible part of the molecule stabilized by a molecular partner [73].

The structure of m153 another member of the m145 family, with unknown function, has also been determined [74] (see Figure 3, Table 1). It was expressed and crystallized in the absence of β2m, which is not required for its cell surface expression. m153 is a noncovalently associated homodimer, not only in its crystal form but also as a purified protein and as expressed at the cell surface. m153 dimerizes in a head-to-tail fashion. Its aminoterminus is somewhat longer than that of classical MHC-I molecules and is tethered to its extended H2b helix via a disulfide bond. Another novel disulfide bond closes the loop connecting two β strands, and a third disulfide, similarly positioned to that of classical MHC-I, is in the α3 domain. Like m144, it has a narrow potential binding groove, not apparently large enough to engage a peptide ligand. The tight juxtaposition of the α1 and α2 helices exposes a significant portion of the β sheet floor. A coiled region separates the amino from the carboxyl-terminal parts of the α2 helix. Although the function of m153 remains as a conundrum, reporter cells constructed with the m153 extracellular domains indicate that some subsets of murine lymphoid cells ligate m153 and activate the reporter through this interaction [84]. Sequence alignment of m153 protein from different MCMV isolates identifies a conserved motif suggestive of an unchanging specific function [74, 87]. Although a definitive function for m153 has yet to be identified, m153 may play an important role in the viral life cycle as it is expressed early in infection and accumulates at the cell surface [84].

m157, a 37 kD surface GPI-linked glycoprotein that is not required for viral replication *in vitro*, is the only known CMV-encoded cell surface molecule that can engage both NK activating (Ly49H) and inhibitory receptors (Ly49I) [56, 72]. The structure of m157 [64] showcases a recognizable MHC-I fold with neither peptide binding groove nor β2m association and a compactness enhanced by extensive intramolecular interactions. m157, like m153, has an extended aminoterminus, but for m157 this is a unique helical region, designated α0 (see Figure 3, Table 1). As with m144 and m153, the α1 juxtaposition to α2 precludes binding to a peptide antigen. Two intrachain disulfide bonds stabilize the $\alpha1\alpha2$ domain, and the α3 domain has a disulfide as well. α2 is joined to α3 by an extended H2b helix, similar in conformation to that of m153. Mutagenesis and binding analysis suggest that m157 engages its Ly49H or Ly49I ligands through a surface distinct from that by which the homologous Ly49A binds to its H-2D^d ligand.

The HCMV UL18 is closer in structure to classical MHC-I molecules and to m144 than it is to m153 or m157 despite the fact that it is only about 25% identical in sequence to MHC-I. UL18 requires peptide and β2m for proper folding [88], and binds the host inhibitory receptor LIR-1 with high affinity [75]. The $\alpha1\alpha2$ domain preserves the highly conserved disulfide of MHC-I molecules, and also has a canonical disulfide bridge in the α3 domain. In addition, it links two adjacent β strands of α3 with another disulfide (see Figure 3, Table 1). Both α3 domain disulfides are necessary for proper association with the LIR-1 ligand [89, 90].

The three-dimensional structure of the 2L protein, another MHC-I homologue [85] of the human tanapox

Figure 2: Amino acid sequence alignments of the extracellular domains of classical MHC-I molecules from mouse (H-2D^d) and human (HLA-A2) with MHC-I-like viral immunoevasins were generated using ClustalW module of MacVector 10.6.6. H-2D^d and HLA-A2 share significant similarity at 81.7% (a). UL18 and m144 show detectable sequence similarity (21–40%) with HLA-A2 and H-2D^d. Conserved cysteine residues are in yellow (b). The alignment of m145 family members and other MHC-I-like immunoevasins shows 6–40% sequence similarity to the canonical MHC-I molecules (c).

Table 1: (a) Subunit composition of MHC-I and MHC-I-like viral molecules. (b) Structural differences among MHC-I and MHC-I-like viral molecules.

(a)

Molecule	β2m	Bound peptide ligand
Classical MHC-I (e.g., HLA-A2, H-2D^d)	yes	yes
Viral MHC-I-like molecule		
UL18	yes	yes
m144	yes	no
m153	no	no
m157	no	no
2L	no	no

(b)

Molecule	Peptide binding groove	N-terminal extension	H2b helix	Canonical -S-S-		Non-canonical -S-S-	
				α1α2 domain β5 → α2	α3 domain β2 → β6	α1α2 domain	α3 domain
Classical MHC-I (e.g., HLA-A2, H-2D^d)	yes	no	short	yes	yes	no	no
Viral MHC-I-like							
UL18	yes	no	short	yes	yes	no	β4 → β5
m144	no	no	short	yes	yes	β2 → α1	no
m153	no	Strand, coil	long	no	yes	β4 → loop 5	N-term → H2b
m157	no	α0 helix	long	no	yes	Loop 4	β5 → β6
2L	no	no	no	no	yes	no	C-term loop

virus, has also been determined [77]. Tanapox is a *Yatapoxvirus*, only distantly related to the *Herpesviridae* to which the CMVs belong. The 2L molecule binds TNF-α, in a high-affinity interaction that accounts for inhibition of immune function such as TNF-mediated cellular cytotoxicity. The X-ray structure of the complex of 2L with TNF-α reveals a molecule that lacks the typical MHC-I peptide binding groove. The amino-terminal parts of the α1 and α2 helices are displaced toward the opposite helix, closing the groove. One disulfide bond links the β strand floor to the α2 helix like classical MHC-I molecules, while two others stabilize the α3 domain (see Figure 3, Table 1). The site of interaction between 2L and TNF-α is a large and complementary interface that includes residue of both the α2 and α3 domains of 2L. Thus, 2L preserves the basic MHC-I fold, lacks peptide or β2m, and interacts with the trimeric TNF-α in a novel way.

4. Viral MHC-I-Like Gene Evolution

CMVs and their respective hosts have coevolved, and the origin of the most recent common root for the three families of the *Herpesviridae* (i.e., the α, β, and γ-*Herpesvirinae*) has been estimated to have occurred about 400 million years ago (Ma) [91]. Under the selection of the host immune response, the virus has developed biological solutions for its continued survival. Although a conserved core of genes is observed for the herpesvirus genomes [92], there exist a number of genes, homologous to those of the host [93] which appear to have originated in the host and to have been acquired by lateral (horizontal) transmission. There are many viral genes that on initial evaluation exhibit a very low level of nucleic acid sequence similarity to host genes, but whose ORFs likely encode proteins similar to those of the host. Even more distantly related viral genes are observed, some of which encode proteins that have little or no amino acid sequence similarity to proteins from their hosts, but whose relationship to host proteins may be deduced through various secondary structure threading programs such as 3D-PSSM [94, 95] or phyre [96, 97]. The viral MHC-I-like proteins fall into this latter category, revealing amino acid sequence identity as low as 6%. The evolutionary origin of many of these proteins and their encoding genes, although they seem to have been derived from the host, remains unclear, and efforts to understand their origin rely not only on nucleic acid and protein sequence comparison, but also on a knowledge of the function and structure of the expressed proteins. With the goal of understanding the function and evolution of these genes, several laboratories have determined the three-dimensional structure of representative viral MHC-I-like molecules, and the comparisons that we have summarized above confirm that m144, UL18, m153, m157 of the CMV family, and 2L of the more distantly related tanapox virus all clearly have structural features in common (see Table 1). The structural similarity of each of these proteins to other MHC-I, for example, H-2D^d [86] and MHC-I-like molecules is established not only by an intuitive sense based on the similarity of the location and orientation of secondary structural elements, it is strongly

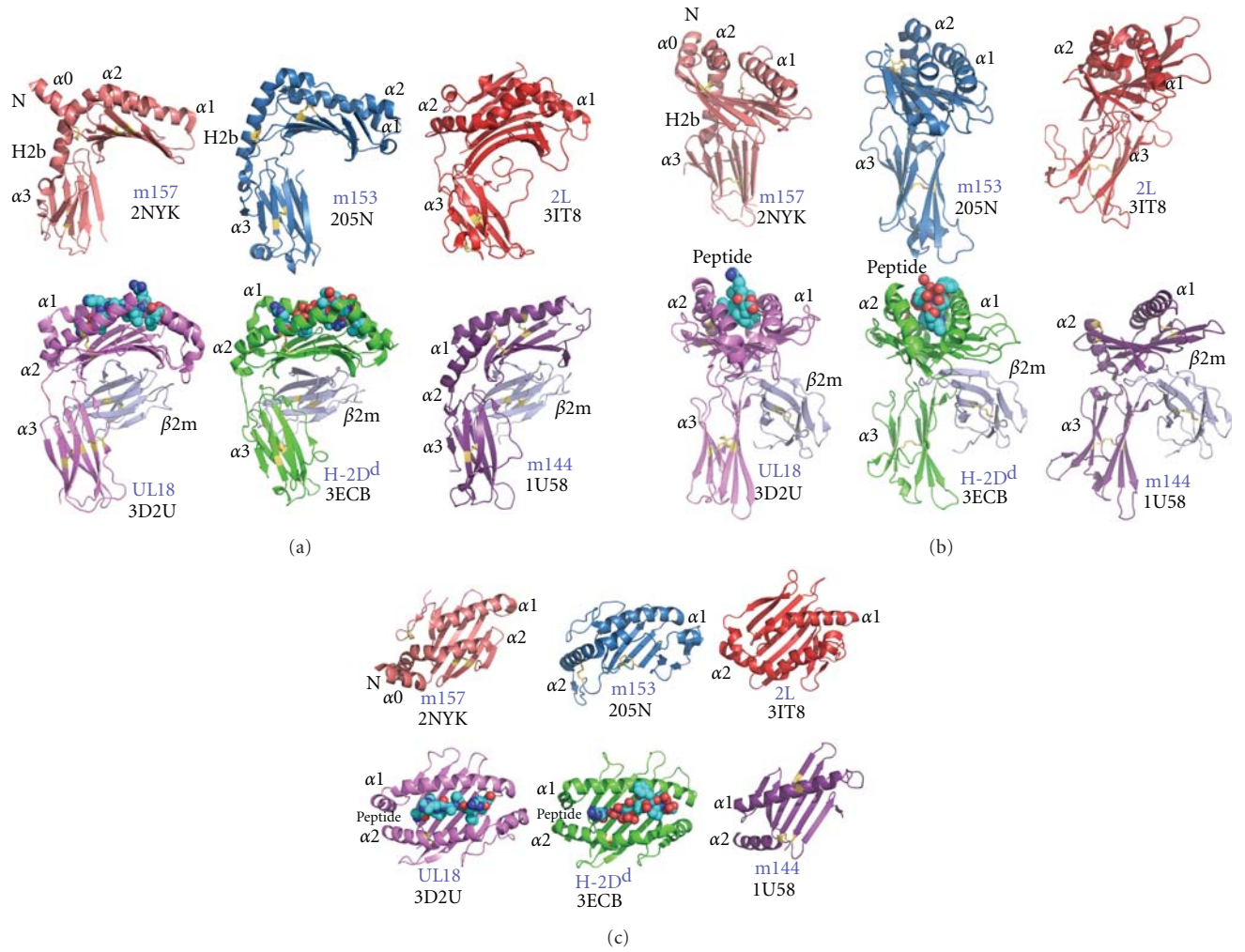

Figure 3: X-ray structures of m157 (2NYK) [64], m153 (205N) [74, 84], m144 (IU58), HCMV UL18 (3D2U) [75], Tanapox 2L protein (3IT8) [85], and H-2D^d (3ECB) [86] reveal both shared and unique features. The disulfide bonds are in yellow. The α1, α2 and H2b helices and α3 domain are labeled. Ribbon diagrams of the structures in (a), rotated 90° to the right in (b), reveal differences in β2m and peptide binding (a, b). The view from the top shows differences in the peptide binding pocket (c). Illustrations were prepared from the superposed structures of the molecules using PyMOL http://www.pymol.org/.

confirmed by quantitative computational superpositions of the crystallographic structures calculated with programs such as Dali [98], Pymol [99], and lsqkab [100].

Thus, arguments for the relationship of these representative proteins and their encoding genes can be made forcefully. In addition, particularly among the rodent members of the 145 family, the amino acid sequence similarities support the notion of a common ancestor. The most difficult problem is whether or not a single evolutionary event, in which a gene encoding a vertebrate MHC-I-like molecule was captured by a single large DNA virus as much as 400 Ma, has given rise to the genes that encode MHC-I-like molecules identifiable in a number of viral species, or whether several independent capture events have occurred for different viruses. The observation that the HCMV protein UL18 and the MCMV protein m144 appear to be closer in structure to classical MHC-I molecules and that the other MCMV proteins, m153 and m157, are more distantly related, favors a single ancient origin. Whether such a hypothesis can withstand the identification, amino acid sequence, and structural analysis of previously unidentified CMV and other viral immunoevasins related to classical MHC-I molecules remains to be determined.

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References

[1] M. J. Reddehase, "Antigens and immunoevasins: opponents in cytomegalovirus immune surveillance," *Nature Reviews Immunology*, vol. 2, no. 11, pp. 831–844, 2002.

[2] D. J. McGeoch, S. Cook, A. Dolan, F. E. Jamieson, and E. A. R. Telford, "Molecular phylogeny and evolutionary timescale for the family of mammalian herpesviruses," *Journal of Molecular Biology*, vol. 247, no. 3, pp. 443–458, 1995.

[3] H. Hengel, U. Reusch, A. Gutermann et al., "Cytomegaloviral control of MHC class I function in the mouse," *Immunological Reviews*, vol. 168, pp. 167–176, 1999.

[4] M. M. Crumpler, K. Y. Choi, M. A. McVoy, and M. R. Schleiss, "A live guinea pig cytomegalovirus vaccine deleted of three putative immune evasion genes is highly attenuated but remains immunogenic in a vaccine/challenge model of congenital cytomegalovirus infection," *Vaccine*, vol. 27, no. 31, pp. 4209–4218, 2009.

[5] W. T. London, A. J. Martinez, and S. A. Houff, "Experimental congenital disease with simian cytomegalovirus in rhesus monkeys," *Teratology*, vol. 33, no. 3, pp. 323–331, 1986.

[6] G. B. Baskin, "Disseminated cytomegalovirus infection in immunodeficient rhesus monkeys," *American Journal of Pathology*, vol. 129, no. 2, pp. 345–352, 1987.

[7] V. DeFilippis and K. Früh, "Rhesus cytomegalovirus particles prevent activation of interferon regulatory factor 3," *Journal of Virology*, vol. 79, no. 10, pp. 6419–6431, 2005.

[8] C. Powers and K. Früh, "Rhesus CMV: an emerging animal model for human CMV," *Medical Microbiology and Immunology*, vol. 197, no. 2, pp. 109–115, 2008.

[9] K. M. Lockridge, G. Sequar, S. S. Zhou, Y. Yue, C. P. Mandell, and P. A. Barry, "Pathogenesis of experimental rhesus cytomegalovirus infection," *Journal of Virology*, vol. 73, no. 11, pp. 9576–9583, 1999.

[10] M. Miller-Kittrell, J. Sai, M. Penfold, A. Richmond, and T. E. Sparer, "Functional characterization of chimpanzee cytomegalovirus chemokine, vCXCL-1(CCMV)," *Virology*, vol. 364, no. 2, pp. 454–465, 2007.

[11] A. J. Davison, A. Dolan, P. Akter et al., "The human cytomegalovirus genome revisited: comparison with the chimpanzee cytomegalovirus genome," *Journal of General Virology*, vol. 84, no. 1, pp. 17–28, 2003.

[12] V. Misra and J. B. Hudson, "Minor base sequence differences between the genomes of two strains of murine cytomegalovirus differing in virulence," *Archives of Virology*, vol. 64, no. 1, pp. 1–8, 1980.

[13] M. Pyzik, A. Kielczewska, and S. M. Vidal, "NK cell receptors and their MHC class I ligands in host response to cytomegalovirus: insights from the mouse genome," *Seminars in Immunology*, vol. 20, no. 6, pp. 331–342, 2008.

[14] M. G. Smith, "Propagation of salivary gland virus of the mouse in tissue cultures," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 86, no. 3, pp. 435–440, 1954.

[15] A. Krmpotic, I. Bubic, B. Polic, P. Lucin, and S. Jonjic, "Pathogenesis of murine cytomegalovirus infection," *Microbes and Infection*, vol. 5, no. 13, pp. 1263–1277, 2003.

[16] C. Powers, V. DeFilippis, D. Malouli, and K. Früh, "Cytomegalovirus immune evasion," *Current Topics in Microbiology and Immunology*, vol. 325, pp. 333–359, 2008.

[17] M. J. Cannon, D. S. Schmid, and T. B. Hyde, "Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection," *Reviews in Medical Virology*, vol. 20, no. 4, pp. 202–213, 2010.

[18] M. R. Schleiss, "Nonprimate models of congenital cytomegalovirus (CMV) infection: gaining insight into pathogenesis and prevention of disease in newborns," *ILAR Journal*, vol. 47, no. 1, pp. 65–72, 2006.

[19] J. S. Orange, M. S. Fassett, L. A. Koopman, J. E. Boyson, and J. L. Strominger, "Viral evasion of natural killer cells," *Nature Immunology*, vol. 3, no. 11, pp. 1006–1012, 2002.

[20] M. J. Reddehase, W. Mutter, K. Münch, H. J. Bühring, and U. H. Koszinowski, "CD8-positive T lymphocytes specific for murine cytomegalovirus immediate-early antigens mediate protective immunity," *Journal of Virology*, vol. 61, no. 10, pp. 3102–3108, 1987.

[21] X. He, H. Yoshida, Y. Minamishima, and K. Nomoto, "Analysis of the role of CD4+ T-cells during murine cytomegalovirus infection in different strains of mice," *Virus Research*, vol. 36, no. 2-3, pp. 233–245, 1995.

[22] E. Wiertz, A. Hill, D. Tortorella, and H. Ploegh, "Cytomegaloviruses use multiple mechanisms to elude the host immune response," *Immunology Letters*, vol. 57, no. 1–3, pp. 213–216, 1997.

[23] W. D. Rawlinson, H. E. Farrell, and B. G. Barrell, "Analysis of the complete DNA sequence of murine cytomegalovirus," *Journal of Virology*, vol. 70, no. 12, pp. 8833–8849, 1996.

[24] L. Brocchieri, T. N. Kledal, S. Karlin, and E. S. Mocarski, "Predicting coding potential from genome sequence: application to betaherpesviruses infecting rats and mice," *Journal of Virology*, vol. 79, no. 12, pp. 7570–7596, 2005.

[25] S. F. Altschul, T. L. Madden, A. A. Schäffer et al., "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs," *Nucleic Acids Research*, vol. 25, no. 17, pp. 3389–3402, 1997.

[26] D. G. Kavanagh, U. H. Koszinowski, and A. B. Hill, "The murine cytomegalovirus immune evasion protein m4/gp34 forms biochemically distinct complexes with class I MHC at the cell surface and in a pre-golgi compartment," *Journal of Immunology*, vol. 167, no. 7, pp. 3894–3902, 2001.

[27] M. F. Kleijnen, J. B. Huppa, P. Lucin et al., "A mouse cytomegalovirus glycoprotein, gp34, forms a complex with folded class I MHC molecules in the ER which is not retained but is transported to the cell surface," *The EMBO Journal*, vol. 16, no. 4, pp. 685–694, 1997.

[28] K. Natarajan, N. Dimasi, J. Wang, D. H. Margulies, and R. A. Mariuzza, "MHC class I recognition by Ly49 natural killer cell receptors," *Molecular Immunology*, vol. 38, no. 14, pp. 1023–1027, 2002.

[29] K. Natarajan, N. Dimasi, J. Wang, R. A. Mariuzza, and D. H. Margulies, "Structure and function of natural killer cell receptors: multiple molecular solutions to self, nonself discrimination," *Annual Review of Immunology*, vol. 20, pp. 853–885, 2002.

[30] J. C. Sun and L. L. Lanier, "The natural selection of herpesviruses and virus-specific NK cell receptors," *Viruses*, vol. 1, no. 3, p. 362, 2009.

[31] S. Bahram, "MIC genes: from genetics to biology," *Advances in Immunology*, vol. 76, pp. 1–60, 2000.

[32] D. Cosman, J. Müllberg, C. L. Sutherland et al., "ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor," *Immunity*, vol. 14, no. 2, pp. 123–133, 2001.

[33] C. Dunn, N. J. Chalupny, C. L. Sutherland et al., "Human cytomegalovirus glycoprotein UL16 causes intracellular sequestration of NKG2D ligands, protecting against natural killer cell cytotoxicity," *Journal of Experimental Medicine*, vol. 197, no. 11, pp. 1427–1439, 2003.

[34] C. L. Sutherland, N. Jan Chalupny, and D. Cosman, "The UL16-binding proteins, a novel family of MHC class I-related ligands for NKG2D, activate natural killer cell functions," *Immunological Reviews*, vol. 181, pp. 185–192, 2001.

[35] A. B. H. Bakker, R. M. Hoek, A. Cerwenka et al., "DAP12-deficient mice fail to develop autoimmunity due to impaired antigen priming," *Immunity*, vol. 13, no. 3, pp. 345–353, 2000.

[36] L. N. Carayannopoulos, O. V. Naidenko, D. H. Fremont, and W. M. Yokoyama, "Cutting edge: murine UL16-binding protein-like transcript 1: a newly described transcript encoding a high-affinity ligand for murine NKG2D," *Journal of Immunology*, vol. 169, no. 8, pp. 4079–4083, 2002.

[37] L. N. Carayannopoulos, O. V. Naidenko, J. Kinder, E. L. Ho, D. H. Fremont, and W. M. Yokoyama, "Ligands for murine NKG2D display heterogeneous binding behavior," *European Journal of Immunology*, vol. 32, no. 3, pp. 597–605, 2002.

[38] A. Cerwenka, A. B. H. Bakker, T. McClanahan et al., "Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice," *Immunity*, vol. 12, no. 6, pp. 721–727, 2000.

[39] A. Diefenbach, A. M. Jamieson, S. D. Liu, N. Shastri, and D. H. Raulet, "Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages," *Nature Immunology*, vol. 1, no. 2, pp. 119–126, 2000.

[40] L. L. Lanier, "DAP10- and DAP12-associated receptors in innate immunity," *Immunological Reviews*, vol. 227, no. 1, pp. 150–160, 2009.

[41] S. Gilfillan, E. M. Ho, M. Cella, W. M. Yokohama, and M. Colonna, "NKG2D recriuts two distinct adapters to trigger NK cell activation and costimulation," *Nature Immunology*, vol. 3, no. 12, pp. 1150–1155, 2002.

[42] A. Diefenbach, E. Tomasello, M. Lucas et al., "Selective associations with signaling proteins determine stimulatory versus costimulatory activity of NKG2D," *Nature Immunology*, vol. 3, no. 12, pp. 1142–1149, 2002.

[43] J. L. Upshaw, L. N. Arneson, R. A. Schoon, C. J. Dick, D. D. Billadeau, and P. J. Leibson, "NKG2D-mediated signaling requires a DAP10-bound Grb2-Vav1 intermediate and phosphatidylinositol-3-kinase in human natural killer cells," *Nature Immunology*, vol. 7, no. 5, pp. 524–532, 2006.

[44] M. Lodoen, K. Ogasawara, J. A. Hamerman et al., "NKG2D-mediated natural killer cell protection against cytomegalovirus is impaired by viral gp40 modulation of retinoic acid early inducible 1 gene molecules," *Journal of Experimental Medicine*, vol. 197, no. 10, pp. 1245–1253, 2003.

[45] A. Krmpotíc, D. H. Busch, I. Bubíc et al., "MCMV glycoprotein gp40 confers virus resistance to CD8 T cells and NK cells in vivo," *Nature Immunology*, vol. 3, no. 6, pp. 529–535, 2002.

[46] LI. Zhi, J. Mans, M. J. Paskow et al., "Direct interaction of the mouse cytomegalovirus m152/gp40 immunoevasin with RAE-1 isoforms," *Biochemistry*, vol. 49, no. 11, pp. 2443–2453, 2010.

[47] A. Krmpotic, M. Hasan, A. Loewendorf et al., "NK cell activation through the NKG2D ligand MULT-1 is selectively prevented by the glycoprotein encoded by mouse cytomegalovirus gene m145," *Journal of Experimental Medicine*, vol. 201, no. 2, pp. 211–220, 2005.

[48] M. Hasan, A. Krmpotic, Z. Ruzsics et al., "Selective down-regulation of the NKG2D ligand H60 by mouse cytomegalovirus m155 glycoprotein," *Journal of Virology*, vol. 79, no. 5, pp. 2920–2930, 2005.

[49] J. D. Mintern, E. J. Klemm, M. Wagner et al., "Viral interference with B7-1 costimulation: a new role for murine cytomegalovirus Fc receptor-1," *Journal of Immunology*, vol. 177, no. 12, pp. 8422–8431, 2006.

[50] J. Arapović, T. Lenac, R. Antulov et al., "Differential susceptibility of RAE-1 isoforms to mouse cytomegalovirus," *Journal of Virology*, vol. 83, no. 16, pp. 8198–8207, 2009.

[51] H. G. Ljunggren and K. Karre, "In search of the 'missing self': MHC molecules and NK cell recognition," *Immunology Today*, vol. 11, no. 7, pp. 237–244, 1990.

[52] K. Natarajan, L. F. Boyd, P. Schuck, W. M. Yokoyama, D. Eilat, and D. H. Margulies, "Interaction of the NK cell inhibitory receptor Ly49A with H-2D(d): identification of a site distinct from the TCR site," *Immunity*, vol. 11, no. 5, pp. 591–601, 1999.

[53] J. Tormo, K. Natarajan, D. H. Margulles, and R. A. Mariuzza, "Crystal structure of a lectin-like natural killer cell receptor bound to its MHC class I ligand," *Nature*, vol. 402, no. 6762, pp. 623–631, 1999.

[54] W. Held and D. H. Raulet, "Ly49A transgenic mice provide evidence for a major histocompatibility complex-dependent education process in natural killer cell development," *Journal of Experimental Medicine*, vol. 185, no. 12, pp. 2079–2088, 1997.

[55] A. H. Jonsson, L. Yang, S. Kim, S. M. Taffner, and W. M. Yokoyama, "Effects of MHC class I alleles on licensing of Ly49A+ NK cells," *Journal of Immunology*, vol. 184, no. 7, pp. 3424–3432, 2010.

[56] H. Arase, E. S. Mocarski, A. E. Campbell, A. B. Hill, and L. L. Lanier, "Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors," *Science*, vol. 296, no. 5571, pp. 1323–1326, 2002.

[57] I. Bubić, M. Wagner, A. Krmpotić et al., "Gain of virulence caused by loss of a gene in murine cytomegalovirus," *Journal of Virology*, vol. 78, no. 14, pp. 7536–7544, 2004.

[58] J. R. Carlyle, A. Mesci, J. H. Fine et al., "Evolution of the Ly49 and Nkrp1 recognition systems," *Seminars in Immunology*, vol. 20, no. 6, pp. 321–330, 2008.

[59] V. Voigt, C. A. Forbes, J. N. Tonkin et al., "Murine cytomegalovirus m157 mutation and variation leads to immune evasion of natural killer cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 23, pp. 13483–13488, 2003.

[60] A. J. Corbett, J. D. Coudert, C. A. Forbes, and A. A. Scalzo, "Functional consequences of natural sequence variation of murine cytomegalovirus m157 for Ly49 receptor specificity and NK cell activation," *Journal of Immunology*, vol. 186, no. 3, pp. 1713–1722, 2011.

[61] M.-P. Desrosiers, A. Kielczewska, J.-C. Loredo-Osti et al., "Epistasis between mouse Klra and major histocompatibility complex class I loci is associated with a new mechanism of natural killer cell-mediated innate resistance to cytomegalovirus infection," *Nature Genetics*, vol. 37, no. 6, pp. 593–599, 2005.

[62] A. Kielczewska, M. Pyzik, T. Sun et al., "Ly49P recognition of cytomegalovirus-infected cells expressing H2-D and CMV-encoded m04 correlates with the NK cell antiviral response," *Journal of Experimental Medicine*, vol. 206, no. 3, pp. 515–523, 2009.

[63] L. L. Lanier, "Evolutionary struggles between NK cells and viruses," *Nature Reviews Immunology*, vol. 8, no. 4, pp. 259–268, 2008.

[64] E. J. Adams, Z. S. Juo, R. T. Venook et al., "Structural elucidation of the m157 mouse cytomegalovirus ligand for Ly49 natural killer cell receptors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 24, pp. 10128–10133, 2007.

[65] T. L. Chapman, A. P. Heikema, and P. J. Bjorkman, "The inhibitory receptor LIR-1 uses a common binding interaction to recognize class I MHC molecules and the viral homolog UL18," *Immunity*, vol. 11, no. 5, pp. 603–613, 1999.

[66] C. A. O'Callaghan, A. Cerwenka, B. E. Willcox, L. L. Lanier, and P. J. Bjorkman, "Molecular competition for NKG2D: H60 and RAE1 compete unequally for NKG2D with dominance of H60," *Immunity*, vol. 15, no. 2, pp. 201–211, 2001.

[67] A. H. Davis, N. V. Guseva, B. L. Ball, and J. W. Heusel, "Characterization of murine cytomegalovirus ml57 from infected cells and identification of critical residues mediating recognition by the NK cell receptor Ly49H," *Journal of Immunology*, vol. 181, no. 1, pp. 265–275, 2008.

[68] J. Wang, M. C. Whitman, K. Natarajan, J. Tormo, R. A. Mariuzza, and D. H. Margulies, "Binding of the natural killer cell inhibitory receptor Ly49A to its major histocompatibility complex class I ligand: crucial contacts include both H-2D and β-microglobulin," *Journal of Biological Chemistry*, vol. 277, no. 2, pp. 1433–1442, 2002.

[69] J. Dam, R. Guan, K. Natarajan et al., "Variable MHC class I engagement by Ly49 natural killer cell receptors demonstrated by the crystal structure of Ly49C bound to H-2K," *Nature Immunology*, vol. 4, no. 12, pp. 1213–1222, 2003.

[70] B. K. Kaiser, J. C. Pizarro, J. Kerns, and R. K. Strong, "Structural basis for NKG2A/CD94 recognition of HLA-E," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 18, pp. 6696–6701, 2008.

[71] S. Beck and B. G. Barrell, "Human cytomegalovirus encodes a glycoprotein homologous to MHC class-I antigens," *Nature*, vol. 331, no. 6153, pp. 269–272, 1988.

[72] H. R. C. Smith, J. W. Heusel, I. K. Mehta et al., "Recognition of a virus-encoded ligand by a natural killer cell activation receptor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 13, pp. 8826–8831, 2002.

[73] K. Natarajan, A. Hicks, J. Mans et al., "Crystal structure of the murine cytomegalovirus MHC-I homolog m144," *Journal of Molecular Biology*, vol. 358, no. 1, pp. 157–171, 2006.

[74] J. Mans, K. Natarajan, A. Balbo et al., "Cellular expression and crystal structure of the murine cytomegalovirus major histocompatibility complex class I-like glycoprotein, m153," *Journal of Biological Chemistry*, vol. 282, no. 48, pp. 35247–35258, 2007.

[75] Z. Yang and P. J. Bjorkman, "Structure of UL18, a peptide-binding viral MHC mimic, bound to a host inhibitory receptor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 29, pp. 10095–10100, 2008.

[76] F. Yang, A. P. West, and P. J. Bjorkman, "Crystal structure of a hemojuvelin-binding fragment of neogenin at 1.8Å," *Journal of Structural Biology*, vol. 174, no. 1, pp. 239–244, 2011.

[77] Z. Yang, A. P. West, and P. J. Bjorkman, "Crystal structure of TNFα complexed with a poxvirus MHC-related TNF binding protein," *Nature Structural and Molecular Biology*, vol. 16, no. 11, pp. 1189–1191, 2009.

[78] B. E. Gewurz, R. Gaudet, D. Tortorella, E. W. Wang, H. L. Ploegh, and D. C. Wiley, "Antigen presentation subverted: structure of the human cytomegalovirus protein US2 bound to the class I molecule HLA-A2," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 12, pp. 6794–6799, 2001.

[79] S. Müller, G. Zocher, A. Steinle, and T. Stehle, "Structure of the HCMV UL16-MICB complex elucidates select binding of a viral immunoevasin to diverse NKG2D ligands," *PLoS Pathogens*, vol. 6, no. 1, Article ID e1000723, 2010.

[80] H. E. Farrell, H. Vally, D. M. Lynch et al., "Inhibition of natural killer cells by a cytomegalovirus MHC class I homologue in vivo," *Nature*, vol. 386, no. 6624, pp. 510–514, 1997.

[81] E. Cretney, M. A. Degli-Esposti, E. H. Densley, H. E. Farrell, N. J. Davis-Poynter, and M. J. Smyth, "m144, a murine cytomegalovirus (MCMV)-encoded major histocompatibility complex class I homologue, confers tumor resistance to natural killer cell-mediated rejection," *Journal of Experimental Medicine*, vol. 190, no. 3, pp. 435–443, 1999.

[82] J. Westbrook, Z. Feng, S. Jain et al., "The protein data bank: unifying the archive," *Nucleic Acids Research*, vol. 30, no. 1, pp. 245–248, 2002.

[83] T. L. Chapman and P. J. Bjorkman, "Characterization of a murine cytomegalovirus class I major histocompatibility complex (MHC) homolog: comparison to MHC molecules and to the human cytomegalovirus MHC homolog," *Journal of Virology*, vol. 72, no. 1, pp. 460–466, 1998.

[84] J. Mans, *Characterization of mouse cytomegalovirus MHC-I homologs*, Doctoral Dissertation, Faculty of Health Scuences, University of Witwatersrand, Johannesburg, South Africa, 2008.

[85] M. M. Rahman, D. Jeng, R. Singh, J. Coughlin, K. Essani, and G. McFadden, "Interaction of human TNF and β2-microglobulin with Tanapox virus-encoded TNF inhibitor, TPV-2L," *Virology*, vol. 386, no. 2, pp. 462–468, 2009.

[86] R. Wang, K. Natarajan, and D. H. Margulies, "Structural basis of the CD8$\alpha\beta$/MHC class I interaction: focused recognition orients CD8β to a T cell proximal position," *Journal of Immunology*, vol. 183, no. 4, pp. 2554–2564, 2009.

[87] J. Mans, L. I. Zhi, M. J. R. Revilleza et al., "Structure and function of murine cytomegalovirus MHC-I-like molecules: how the virus turned the host defense to its advantage," *Immunologic Research*, vol. 43, no. 1-3, pp. 264–279, 2009.

[88] M. L. Fahnestock, J. L. Johnson, R. M. Renny Feldman, J. M. Neveu, W. S. Lane, and P. J. Bjorkman, "The MHC class I homolog encoded by human cytomegalovirus binds endogenous peptides," *Immunity*, vol. 3, no. 5, pp. 583–590, 1995.

[89] C. S. Wagner, A. Rölle, D. Cosman, H. G. Ljunggren, K. D. Berndt, and A. Achour, "Structural elements underlying the high binding affinity of human cytomegalovirus UL18 to leukocyte immunoglobulin-like receptor-1," *Journal of Molecular Biology*, vol. 373, no. 3, pp. 695–705, 2007.

[90] M. Occhino, F. Ghiotto, S. Soro et al., "Dissecting the structural determinants of the interaction between the human cytomegalovirus UL18 protein and the CD85j immune receptor," *Journal of Immunology*, vol. 180, no. 2, pp. 957–968, 2008.

[91] D. J. McGeoch, D. Gatherer, and A. Dolan, "On phylogenetic relationships among major lineages of the Gammaherpesvirinae," *Journal of General Virology*, vol. 86, no. 2, pp. 307–316, 2005.

[92] D. J. McGeoch, A. Dolan, and A. C. Ralph, "Toward a comprehensive phylogeny for mammalian and avian herpesviruses," *Journal of Virology*, vol. 74, no. 22, pp. 10401–10406, 2000.

[93] M. Raftery, A. Muller, and G. Schonrich, "Herpesvirus homologues of cellular genes," *Virus Genes*, vol. 21, no. 1-2, pp. 65–75, 2000.

[94] P. A. Bates, L. A. Kelley, R. M. MacCallum, and M. J. E. Sternberg, "Enhancement of protein modeling by human intervention in applying the automatic programs 3D-JIGSAW and 3D-PSSM," *Proteins: Structure, Function and Genetics*, vol. 45, supplement 5, pp. 39–46, 2001.

[95] L. A. Kelley, R. M. MacCallum, and M. J. E. Sternberg, "Enhanced genome annotation using structural profiles in the program 3D-PSSM," *Journal of Molecular Biology*, vol. 299, no. 2, pp. 499–520, 2000.

[96] L. A. Kelley and M. J. Sternberg, "Protein structure prediction on the Web: a case study using the Phyre server," *Nature Protocols*, vol. 4, no. 3, pp. 363–371, 2009.

[97] R. M. Bennett-Lovsey, A. D. Herbert, M. J. E. Sternberg, and L. A. Kelley, "Exploring the extremes of sequence/structure space with ensemble fold recognition in the program Phyre," *Proteins: Structure, Function and Genetics*, vol. 70, no. 3, pp. 611–625, 2008.

[98] L. Holm and P. Rosenström, "Dali server: conservation mapping in 3D," *Nucleic Acids Research*, vol. 38, no. 2, pp. W545–W549, 2010.

[99] "The PyMOL Molecular Graphics System," V.X.H., Schrödinger, LLC.

[100] N. Collaborative Computational Project, "The CCP4 suite: programs for protein crystallography," *Acta Crystallographica*, vol. D50, pp. 760–763, 1997.

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Review Article

The Impact of Ly49-NK Cell-Dependent Recognition of MCMV Infection on Innate and Adaptive Immune Responses

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Clinical and experimental data indicate that a subset of innate lymphocytes, natural killer (NK) cells, plays a crucial role in the response against herpesviruses, especially cytomegaloviruses (CMV). Indeed, in mice, NK cells, due to the expression of germline encoded Ly49 receptors, possess multiple mechanisms to recognize CMV infection. Classically, this results in NK cell activation and the destruction of the infected cells. More recently, however, this unique host-pathogen interaction has permitted the discovery of novel aspects of NK cell biology, implicating them in the regulation of adaptive immune responses as well as in the development of immunological memory. Here, we will concisely review the newly acquired evidence pertaining to NK cell Ly49-dependent recognition of MCMV-infected cell and the ensuing NK cell regulatory responses.

If one way be better than another, that you may be sure is nature's way.

Aristotle

1. Introduction

Natural killer (NK) cells constitute a unique effector lymphocyte lineage, classically defined as part of the innate immune system. Their importance is well characterized in the context of health, under normal physiological conditions. For instance, NK cells are involved in tumor surveillance, given their natural cytotoxicity against neoplasms. In pregnancy, NK cells account for 70% of decidual leukocytes; they are thought to mediate trophoblast invasion into the uterine lining and the modification of maternal spiral arteries [1]. In addition to their implication in these crucial components of homeostasis and normal development, NK cells are well known for their considerable role in defense against infection.

Infection with a wide variety of pathogens can be limited by the action of NK cells, specifically intracellular microorganisms. Indeed, NK cells play a substantial role in the initial control of both bacterial (e.g., *Listeria monocytogenes*) and parasitic (e.g., *Plasmodium* species) spread [2]. Yet, in the context of infection, the hallmark of NK cells remains their potent antiviral activity. NK cells participate in the clearance of many different viruses (human immunodeficiency virus, coxsackievirus, influenza or poxviruses, etc.), but their contribution is indispensable with regards to infection with members of the *Herpesviridae* family (herpes simplex virus (HHV-1/2), varicella zoster virus (HHV-3), and cytomegalovirus (HHV-5)) [2]. Plentiful evidence supports this, namely, the cases of two young patients suffering from numerous and recurrent herpesviral infections due to their nonfunctional NK cells [3, 4]. In mice, NK cell depletion and adoptive transfer have long been known to increase, respectively, susceptibility and resistance to mouse CMV (MCMV).

The antiviral activity of NK cells relies on the various effector functions induced following their activation. On the

one hand, NK cells secrete several cytokines, such as IFN-γ, MIP1-α/β, RANTES, IL-10, activation-induced T-cell-derived chemokine-related cytokine (ATAC, lymphotactin), and others. On the other hand, NK cells display strong cytotoxicity. Indeed, they can kill virus-infected cells without prior activation, and they do so via a number of mechanisms, namely exocytosis of perforin-granzymes granules and signaling through members of the TNF death receptor family [5]. Yet, cytokine production and cytotoxicity must necessarily be preceded by the recognition of infected cells by NK lymphocytes, a phenomenon that has mostly been studied using a mouse model of infection.

CMVs are prototypical β-subgroup members of the *Herpesviridae* family. Given the nonredundant role played by NK cells in resolving infections with these viruses, it is not surprising that CMVs would be perfect candidates for the study of NK cell responses. Unfortunately, the strict species specificity of CMVs precludes experimental infection of mice with human CMV (HCMV). Yet, MCMV shares many features with its human counterpart, including genome structure and disease manifestations [6]. Both are natural pathogens of their respective host and have coevolved with them for eons. Moreover, both viruses have developed varied and analogous immune-evasion mechanisms that heavily implicate NK cells [7]. Therefore, early MCMV infection has become an established model to study NK cells and, more specifically, their impressive ability to distinguish self from nonself through their germ-line encoded receptors. In addition, at later times post-infection, this model reveled the unforeseen involvement of NK cells in the adaptive immune responses.

NK cells discriminate between infected and healthy cells using an extensive panel of cell surface receptors, both activating and inhibitory. Among the various receptor families involved in this process, Ly49 receptors have proven themselves to be particularly important for MCMV detection by murine NK cells. These polygenic and polymorphic receptors are clustered at the Natural Killer Cell Complex (NKC) on mouse chromosome 6 [8]. They are stochastically expressed as disulfide-linked homodimers primarily on the surface of NK cells, but also on subsets of monocytes, macrophages, dendritic cells (DCs), and T cells [9]. In terms of ligand specificity, inhibitory Ly49 receptors recognize self-MHC class I molecules (MHCI, also called H-2 in mice), whereas their activating counterparts can bind to various protein determinants of infection.

Ly49 receptors are structurally classified as type II transmembrane, C-type lectin-like proteins. Their extracellular domain is comprised of a flexible stalk and a Natural Killer Domain (NKD), which provides ligand binding specificity and is structurally conserved among all members of the Ly49 family. Yet, activating and inhibitory receptors differ with regards to their intracellular domains. Indeed, inhibitory Ly49 receptors possess an immunoreceptor tyrosine-based inhibition motif (ITIM) within their intracellular domain. Conversely, activating Ly49 receptors lack this ITIM motif; instead, a positively charged arginine residue in their transmembrane domain interacts with the DAP12/DAP10 adaptor proteins, which bears an immunoreceptor tyrosine-based activation motif (ITAM).

During infection, Ly49 receptor triggering leads to the initiation of a signaling cascade, the result of which is either inhibition or activation of the NK cell. In the case of both activating and inhibitory receptors, the first step of this cascade is the phosphorylation of the tyrosine residue contained in their respective ITAM or ITIM, most likely by a Src family kinase [5]. This phosphorylation recruits either SHIP-1, SHP-1, or SHP-2 in the case of inhibitory receptors, or Syk, ZAP-70 and PI(3)K or Grb2 for activating receptors. In both cases, numerous downstream effectors are involved. The end result of inhibitory receptor triggering is the dephosphorylation of ITAMs linked to activating NK receptors and the prevention of Ca^{2+} influx, degranulation, cytokine production, and NK cell proliferation. In opposition, activating Ly49 receptor engagement induces the reorganization of the actin cytoskeleton to enable cell polarization and the release of cytolytic granules, as well as the transcription of many cytokine and chemokine genes.

Of note, inhibition is by far the most common signal received by NK cells over the course of their lifetime. Moreover, during NK cell development, binding of inhibitory Ly49 receptors to self-MHCI molecules renders them functional, that is to say capable of cytokine secretion (e.g., IFN-γ) and cytotoxic killing [10]. This model, called NK cell education or licensing, postulates that the stronger the inhibitory Ly49-self MHCI interactions are, the more competent the NK cell will emerge. The immune response which follows MCMV infection relies on the interplay of soluble factors and numerous cell types, among them NK cells. In the early innate response, Ly49 receptors are critical for NK cell activation, which in turn controls viral proliferation, due to their ability to recognize various viral and self-ligands. Nevertheless, initial NK cell proliferation is Ly49-independent and nonselective, while the subsequent preferential NK cell proliferation is driven by activating Ly49 receptors. At later stages of the infection, NK cell secreted cytokines and apt virus control allows them to regulate the adaptive response. Most surprisingly, Ly49-mediated activation of NK cells also allows the generation of NK cells with several characteristics seen in memory T cells (Figure 2(a)).

All of these aspects of MCMV-Ly49-driven NK cell responses will be examined in this paper. Most importantly, evidence will be presented to highlight the previously unanticipated sophistication of the NK cell response against infection, redefining the established concepts of innate and adaptive immunity.

2. Immediate Nonspecific NK Cells Response to MCMV Infection

From its initial peripheral sites of entry, MCMV spreads to the lymph nodes and via the bloodstream to the spleen, and the liver, infecting a broad spectrum of cell types. Macrophages, hepatocytes, and reticular fibroblasts are some of its primary targets [11, 12]. At 6–8 hours after infection (p.i.), lymphotoxin α/β expressed on the surface of B cells

interacts with its cognate receptor on splenic stromal cells, triggering type I interferon (IFN-α/β) secretion by the latter [13, 14]. Within 24 hours after the initial infection, before the end of the first round of viral division, this first wave of type I IFN secretion wanes. Subsequently, a second round of viral infection occurs, this time in many more organs, making the infection systemic.

Over the course of MCMV infection, several molecular viral byproducts are generated due to the lytic nature of the pathogen. Plasmacytoid DCs (pDCs) engulf infected cell debris and recognize them through their endosomal pattern recognition receptors (PRRs), that is, TLR3, TLR7 and TLR9; this recognition induces a second, much stronger wave of type I IFN secretion (~36 hours p.i) as well as the production of other proinflammatory cytokines (IL-12, IL-6, IL-1β, TNF-α, etc.) [15–17]. In parallel, the presence of MCMV particles in the cytoplasm of pDCs can lead to viral recognition by the NOD-like and RIG-I-like helicase sensors resulting in secretion of more pro-inflammatory cytokines; these molecules probably also contribute to pDC-independent secretion of type I IFN [18–20].

Conventional DCs (cDCs) are one of the many cell types that are initially infected by MCMV. In response to infection, they secrete IL-12, IL-18, IL-15 (trans-presented), and type I IFN all of which are required for NK cell activation. Indeed, different NK cell effector functions require different cytokine inputs for their initiation: while the IFN-$\alpha\beta$/STAT1 pathway is necessary for cytotoxicity and initial proliferation, NK cell IFN-γ production during the first 2 days following infection necessitates IL-18 or IL-12/STAT4 (Figure 2(a)i) [21–23]. Moreover, the IFN-γ secreted by infected cDCs, along with macrophage-secreted MIP1-α, are critical for the control of viral loads in the liver and lungs [24]. IFN-γ is also important for perforin-dependent control of viral replication in the spleen [25]. Once activated, NK cells start to eliminate infected cDCs and TNF-α-secreting macrophages, thus reducing the immunopathology caused by this cytokine [26]. In short, the initial NK cell response against MCMV is mediated by a cytokine storm; it is rapid, nonspecific, and global, involving NK cells with diverse Ly49 repertoires. Yet, it does not effectively control viral spread.

Indeed, despite the high levels of antiviral cytokines present in the serum, MCMV replication proceeds unhindered. This is true for all inbred strains at day 1.5 after infection. Yet, by day 3 p.i., mouse strains can be segregated according to their NK cell-dependent control of MCMV [27]. Most strains are susceptible to the virus, while a select few are resistant. These strains include C57BL/6 (B6) and MA/MyJ mice, whose resistance depends on their strain-specific activating Ly49 receptor, as well as PWK/Pas and NZW/LacJ mice, whose resistance is not yet characterized [27–29].

3. Specific Recognition of MCMV-Infected Cells

3.1. Ly49H/m157 Axis. MCMV resistance in B6 mice depends on the presence of the activating Ly49H receptor on the surface of their NK cells [30–32]. In this mouse strain, approximately 50% of NK cells express the receptor. Ly49H binds directly to m157, a MCMV-encoded glycoprotein with structural homology to host MHCI molecules; unlike the latter, however, m157 is a GPI-anchored protein, does not associate with β_2-microglobulin, or present peptides at the surface of the cell [33–35]. During the early phase of the infection, Ly49H-m157 ligation initiates an activating signaling cascade, resulting in clonal proliferation of Ly49H$^+$ NK cells, killing of infected cells, and secretion of cytokines (IFN-γ, MIP1-α, TNF-α, etc.), all of which result in the reduction of viral loads (Figure 1(a)).

The importance of this receptor-ligand pair in the control of MCMV infection was demonstrated by numerous studies. In the case of the virus, resistant mice became susceptible upon infection with a *m157*-deletant MCMV ($\Delta m157$) (Figure 1(b)) [36]. As for the host, deletion of Ly49H gene or deletion or loss of function mutation of the DAP12 gene were shown to render mice susceptible to MCMV infection, as revealed by high viral titers [37–39]. Conversely, transgenesis of Ly49H into initially susceptible strains was found to make them resistant to MCMV [40].

Once the interaction between Ly49H and m157 was established as an essential component of MCMV resistance in B6 mice, the nature of this interaction was investigated. Structural analysis, molecular modeling, and targeted mutations of both m157 and Ly49H have shown the importance of the receptor homodimerization domain. Indeed, Ly49H receptors carrying mutations in this region display reduced m157 recognition, while mutations in the putative ligand-binding regions had limited impact on this same interaction [41]. In the same vein, mutation of buried residues required for correct m157 folding ($\alpha 0 : \alpha 2$ helix interactions) altered the ability of the viral molecule to bind Ly49H, while mutations in the exposed and scattered residues across the entire m157 structure did not [42]. Furthermore, posttranslational modifications can also affect Ly49H-m157 binding. Indeed, given the four putative N-glycosylation motifs (NXT/S) of m157, a variety of glycosylated isoforms were found to be expressed on the surface of MCMV-infected cells. Although these isoforms are also recognized by Ly49H, their binding stability and half-life are increased as compared to the unglycosylated m157 [43]. These results reveal a quite tolerant recognition of m157 by Ly49H where several amino acid substitutions are necessary in order to disrupt m157-Ly49H interactions. Such a molecular arrangement could allow Ly49H to recognize variable m157 molecules, emerging as the MCMV attempts to escape immune recognition.

From an evolutionary standpoint, capture of the *H-2* gene by MCMV is thought to have initiated a cascade of events leading to the emergence of the Ly49H-m157 interaction. Originally, this capture could have allowed the virus to exploit a MHCI homologue capable of triggering inhibitory Ly49 receptors. Since the Ly49 locus evolves rapidly, any inhibitory receptor could have been converted to an activating one, including those with binding specificity for m157 [44, 45]. In support of this, m157 can be bound by the inhibitory Ly49I receptor from 129/J mice; unfortunately, the involvement of Ly49I in susceptibility to MCMV infection could not be assessed due to a defect in

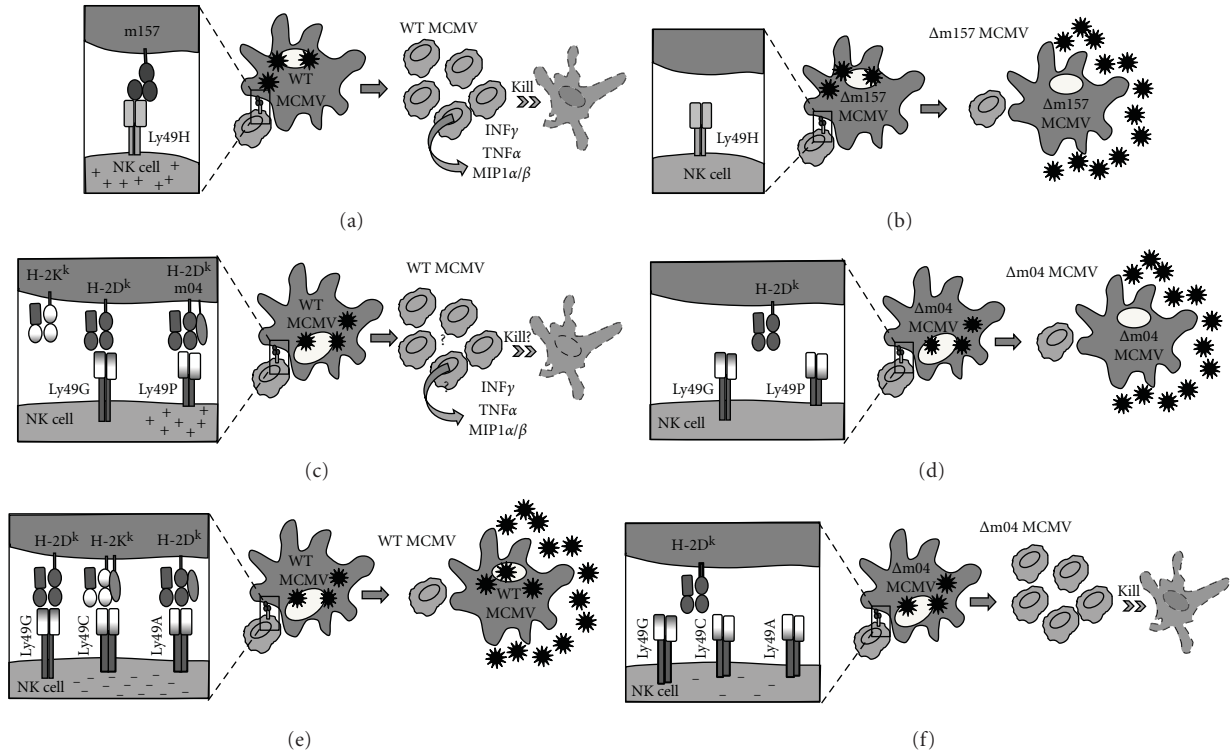

Figure 1: Multiple modes of Ly49-NK cell-dependent recognition of MCMV-infected cell. NK cell-dependent detection of MCMV infection occurs through different Ly49 receptors and viral proteins. (a, b) Ly49H-m157, operating in C57BL/6 mice; (c, d) Ly49P-*m04*:H-2D^k or Ly49G-H-2D^k, operating in MA/MyJ mice; (e, f) Inhibitory Ly49-H-2:*m04*, operating in BALB mice. (a, c) Triggering of the activating Ly49 receptors by their viral ligand, m157, or by their virally modified self-ligands, *m04*:H-2D^k, leads to NK cell cytotoxicity, proliferation, and secretion of cytokines. (b, d) The absence of these ligands prevents NK cells from recognizing and responding to infected cells. (e) However, triggering of inhibitory Ly49 receptors by virally modified self-ligands results in NK cell unresponsiveness during the infection. (f) NK cell responsiveness is restored upon infection with a deletant virus lacking the protein required for modifying self-ligands. WT MCMV: Wild-type MCMV, Δm157: MCMV lacking m157, Δm04: MCMV lacking m04, (+): activation, (-): inhibition.

NK cell signaling within this strain [33]. Recently, additional inhibitory Ly49 receptors, namely, Ly49C from the B6, BALB/c, and NZB strains, have been shown to interact with the m157 obtained from wild-derived MCMV isolates [46]. Interestingly, Ly49C-specific recognition of nonlaboratory strain-MCMV-infected cells did not seem to interfere with Ly49H-mediated NK cell activation and the ensuing antiviral response in B6 mice. The dominant Ly49H recognition of m157 is supported by the fact that sequential passage of wild-type (WT) MCMV in B6 mice leads to the emergence of "NK cell-escape" m157 variants, which evade Ly49H-mediated NK cell recognition [47]. Nevertheless, the m157 proteins of MCMV strains isolated from wild mice are highly variable, indicating that outside of laboratory settings, both inhibitory and activating Ly49 receptors could be targeted by the virus. In any case, the fact that m157, one of many MCMV-encoded proteins, can be recognized by different Ly49 receptors, both activating and inhibitory, suggested that other mechanisms of MCMV recognition might exist in other mouse strains.

3.2. Ly49P/m04/H-2D^k Axis. In this second mechanism of MCMV-infected cell recognition, an activating Ly49 receptor recognizes "altered" self-MHCI molecules rather than a viral MHCI homologue; this alteration of self-MHCI molecules stems from their association with viral protein. This type of recognition could be used by NK cells from MA/MyJ mice, which are resistant to MCMV even though they lack Ly49H. Indeed, MA/MyJ resistance has been associated with a genetic interaction between the NKC and the *H-2^k* loci or the *H-2^k* locus alone [48–50]. At the cellular level, MA/MyJ resistance is correlated with the expression of the activating Ly49P receptor. This receptor can recognize infected cells given the surface expression of two specific elements: the viral *m04*/gp34 protein in association with H-2D^k molecules (Figure 1(c)). In order to dissect the contribution of *H-2* to this recognition, chimeric *H-2* molecules were produced, in which domains were swapped between H-2D^b and H-2D^k; these chimeric molecules were used to show that the H-2D^k-binding platform in particular is required for this recognition. Furthermore, the ectopic expression of *m04*/gp34 alone cannot stimulate Ly49P-bearing reporter cells in the absence of infection. This stimulation, however, is restored when target cells are infected with a viral deletion mutant lacking *m04* (Δ*m04* MCMV), suggesting that another host or viral factor is necessary for Ly49P/*m04*/H-2D^k-mediated recognition of infected cells. *m04*/gp34 is crucial for the *in vivo* NK cell response in MA/MyJ mice, since

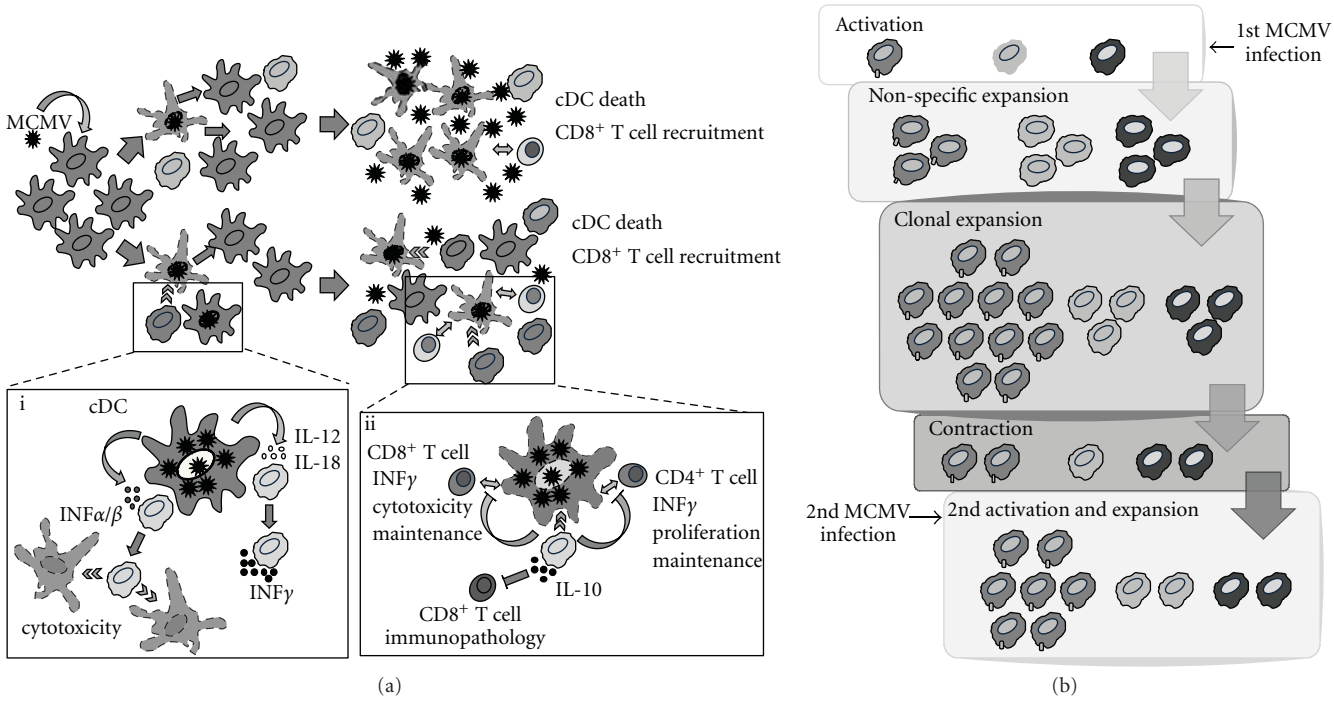

Figure 2: Different regulatory roles of Ly49H$^+$ NK cells in adaptive immune responses. (a) Ly49H$^+$ NK cells rapidly control viral replication and protect cDCs from MCMV infection. This allows for rapid recruitment and priming of CD8$^+$ T cells and reduces viral and self-immunopathology. (i) Interaction of NK cells with MCMV-infected conventional dendritic cells (cDCs) induces the former's proliferation and cytotoxicity via IFN-α/β or secretion of IFN-γ via IL-12/IL-18. (ii) Ly49H$^+$ NK killing of infected cDCs will also reduce the priming of CD4$^+$/CD8$^+$ T cells and dampen the ensuing adaptive immune responses; NK cells will produce IL-10, which will limit excessive CD8$^+$ T cell responses, thus reducing immunopathology. (b) Schematic representation of NK cell proliferative responses upon primary and secondary MCMV infections. ↑: increase, ↓: decrease.

normally resistant MA/MyJ mice are unable to control viral replication following infection with Δ*m04* MCMV [51] (Figure 1(d)).

The importance of H-2D^k in the resistance of MA/MyJ mice was also demonstrated *in vivo*: (1) by the creation of an H-2^k congenic mouse panel; (2) by the transgenesis of H-2D^k into susceptible H-2^b mice. In both cases, the mice became capable of controlling the viral infection, as opposed to H-2^b animals [50, 52, 53]. Xie et al. tested their H-2^k congenic mouse panel further and showed that Ly49G$^+$ NK cells expanded preferentially in these mice at 90 hours p.i. Their resistance to MCMV infection was abrogated following depletion of Ly49G$^+$ NK cells, which suggests that Ly49G is somehow involved [52, 53]. Since an inhibitory receptor cannot directly mediate resistance to MCMV, the authors hypothesized that appropriate licensing via Ly49G:H-2D^k interaction renders NK cells functional, capable of subsequently recognizing and eliminating MCMV through Ly49P/*m04*/H-2D^k (Figure 1(c)). Nevertheless, it remains unclear how or if licensing contributes to the ability of NK cells to resolve MCMV infection. Indeed, it has recently been shown that unlicensed NK cells respond better to MCMV infection [54]. Regardless of the role of licensing, the available evidence suggests that several Ly49 receptors might be involved in the control of MCMV infection in MA/MyJ mice.

3.3. Inhibitory Ly49/m04/MHCI Axis. The contribution of inhibitory Ly49 receptors to the NK cell-mediated control of MCMV infection has recently been evaluated. Indeed, studies using BALB mice show that the presence of Ly49 inhibitory receptors negatively impacts early MCMV resistance, that is, it makes mice susceptible to the virus [55]. BALB mice are considered susceptible, that is to say that they are unable to control the replication of WT MCMV in the spleen at day 3 p.i. However, infecting these mice with Δ*m04* MCMV results in a significant reduction of viral loads in this organ and increased NK cell proliferation in a *H-2*-dependent manner. Indeed, BALB mice of the H-2^k (BALB.K) background showed the greatest reduction in viral load upon infection with Δ*m04* MCMV, whereas none was seen in H-2^b mice (BALB.By); the viral load reduction in H-2^d mice (BALB/c) was an intermediate value between that of H-2^k and H-2^b mice. This reduction in viral load is due to the absence of *m04*/gp34, which maintains surface expression of MHCI molecules, and the action of activating receptors such as NKG2D. As such, in WT MCMV-infected cells, sufficient levels of MHCI molecules are displayed on the cell surface; this allows inhibitory Ly49 receptors to be triggered and to deliver their inhibitory signal, the result of which is the unresponsiveness of NK cells (Figure 1(e)). In the case of Δ*m04* MCMV infection, the expression of MHCI molecules is severely downregulated, allowing NK

cell activation through the "missing-self" mechanism and improved control of viral spread (Figure 1(f)). Furthermore, viral loads were shown to return to high levels following depletion of certain inhibitory Ly49$^+$ NK cell fractions during Δ*m04* MCMV infection of H-2^d BALB mice. This effect was more pronounced following Ly49G$^+$ NK cell depletion than Ly49C$^+$ or Ly49A$^+$ NK cell depletion. In summary altered self-recognition by activating Ly49 receptors, requiring the presence of self-MHCI molecules associated with viral products, implicates involvement of inhibitory Ly49 receptors, which depending on mice strain can have opposite effects. Thus, when the predominant signal is *m04*-dependent activation (e.g., MA/MyJ via Ly49P), *m04* ablation allows enhanced viral replication; however, when the predominant signal is *m04*-dependent inhibition (e.g., BALB via Ly49G/A), absence of *m04* allows enhanced control of virus replication.

In short, these findings suggest that the early, NK cell-dependent control of viral replication is not solely due to activating Ly49 receptors, but rather that it involves a delicate interplay between signals generated by both activating and inhibitory receptors.

4. Beyond Natural Killing: Regulation of Inflammatory and Adaptive Responses by NK Cells

NK cells have classically been thought of as effectors of the antiviral response through their ability to directly eliminate infected cells. However, recent evidence demonstrates that their role extends beyond that of effector lymphocytes into regulators of the inflammatory and adaptive immune response against viral infection. More specifically, Ly49H$^+$ NK cells are essential for the protection and preservation of cDCs, which can themselves stimulate NK cells and potentiate their response [56, 57]. Nevertheless, infection does occur; NK cell-dependent elimination of cDCs and, in particular, macrophages, reduces the secretion of TNF-α, a major contributor to hemophagocytic lymphohistiocytosis syndrome during MCMV infection [26]. In addition, Ly49H-m157-dependent reduction of viral burden decreases the duration and intensity of pDC activation, which limits very high systemic levels of type I IFN and other cytokines that can be detrimental to the host [32, 58–60]. In doing so, NK cells also accelerate the recruitment and expansion of anti-MCMV specific CD8$^+$ T cells, as it was shown that in mice devoid of Ly49H, the appearance of CD8$^+$ T cells is delayed by ~1 day (Figure 2) [57].

Triggering by m157 is essential for Ly49H$^+$ NK cell amplification and maintenance. In the context of MCMV infection, signaling through Ly49H/DAP12 induces NK cell clonal proliferation, ultimately leading to an increase in the Ly49H$^+$ NK cell frequency from 50% to 80% and clearance of the virus in the spleen and liver by day 6 p.i. [32]. Conversely, in Ly49H$^{-/-}$ mice, the overall amount of NK cells declines past uninfected levels, leading to high viral titers in the spleen at the same time point [59]. Another study, by Lee et al., attempted to dissect the role of Ly49H in various aspect of the NK cell response using *Ly49h* or *perforin* 1 (*Prf1*) knockout mice. For instance, Ly49H-expressing *Prf1*$^{-/-}$ mice were susceptible to MCMV infection, even though Ly49H$^+$ NK cells proliferated substantially. Although in these mice the NK cytotoxic function was absent, they survived a dose that killed *Ly49h*$^{-/-}$ *Prf1*$^{-/-}$ double-knockout mice. Therefore, Ly49H expression was protective. This was explained by the fact that Ly49H$^+$ NK cells secreted IL-10, a potent immunosuppressive cytokine capable of limiting CD8$^+$ T cell cytotoxicity as well as IFN-γ and TNF-α secretion (Figure 2(a)ii) [59]. Thus, Ly49H is required for the maintenance of NK cells until late time-points after infection, allowing them to exert a regulatory function over CD8$^+$ T cells and the immunopathology they can cause.

Paradoxically, the presence of activating Ly49 receptors can also be detrimental to the subsequent adaptive immune response by promoting viral persistence. For example, while Ly49H$^+$ mice could clear infected cDCs better than their Ly49H$^-$ counterparts, killing of these antigen-presenting cells (APCs) prevented them from priming naïve, virus-specific T cells [61]. This lack of appropriate T-cell priming in Ly49H$^+$ mice resulted in the reduced activation, cytotoxicity, IFN-γ secretion, and maintenance of CD8$^+$ T cells, as well as the reduced IFN-γ secretion and maintenance of CD4$^+$ T cell, contributing to MCMV persistence in the salivary glands (Figure 2(a)ii). However, MCMV-infected, immature cDCs have also been shown to induce anergy, poor effector functions, and inferior recall responses in CD4$^+$ and CD8$^+$ T cells [62]. Further research is needed to determine the precise effect of cDC clearance in the overall response to the virus. Nevertheless, Ly49H$^+$ NK cell recognition and removal of MCMV-infected cells can unquestionably shape the course of the adaptive immune response.

5. Memory-Like NK Cells

Another unforeseen NK cell role was evidenced by the discovery that, following activation, NK cells acquire features seen in memory T-cell populations. The existence of memory-like NK cells was first hinted at in a study of chemically-induced contact hypersensitivity (CHS). In this model, mice lacking T and B lymphocytes, the classical mediators of CHS, remained able to elicit an inflammatory response through hepatic NK cells, as measured by dermatitis [63]. Sun et al. [64] followed up on this observation using an MCMV model of infection and adoptive transfer into DAP12$^{-/-}$ mice. The investigators noticed that only Ly49H$^+$ NK cells underwent specific clonal expansion before contraction and persisted for up to 70 days p.i. This was rather astonishing as the generally accepted half-life of mature NK cells is 7 to 17 days [65]. Importantly, irrespective of initial transfer numbers the frequency of the MCMV-specific NK-cell response was similar to primary T-cells responses. They also observed that these long-lived, memory-like NK cells were m157-specific and had superior effector responses upon rechallenge with MCMV as compared to naïve NK cells [64].

The existence of memory-like NK cells was also confirmed by transferring cytokine-activated NK cells into naïve hosts and observing that they could still be detected as far as 22 days after transfer [66]. These memory NK cells were found to have similar cytotoxic activity to that of their naïve counterparts. Moreover, they were unable to constitutively produce IFN-γ yet upon restimulation produced significantly more of this cytokine than naïve NK cells. More recently, again in the CHS model, virus-specific memory-like NK cells were generated in response to influenza virus, VSV or HSV-1 infection. In this case, the adoptive transfer of specific, virus-sensitized hepatic NK cells into naive mice improved their survival following infection with a lethal dose of virus; this was dependent on the CXCR6 chemokine receptor expressed on NK cells [67].

All of these experiments have shown that innate immune cells can retain an intrinsic memory of prior activation, a function until now restricted to antigen-specific, adaptive immune lymphocytes. However, most of the aforementioned studies were performed with immunodeficient donor and recipient mice. Given the adaptability of the immune system, it is possible that donor NK cells, once transferred into these recipients, may have taken up more responsibility (i.e., memory status) than they normally would in WT animals in order to compensate for the absence of T and B cells. As for the work performed by Sun et al., even though their donor NK cells came from WT mice, they were transferred into neonate animals, whose adaptive immune responses are arguably as ineffective as that of immunodeficient mice.

6. Conclusions

Since their discovery over 30 years ago, it has become clear that NK cells possess numerous functions, going beyond their original "natural killers of tumors" role. Among these, they act as a first line of defense in the context of infection with a variety of pathogens, in particular viruses. Humans have been coevolving for millions of years with some of these viruses, namely CMVs; therefore, they "know us better than we know ourselves", having shaped our immune system and been shaped in return.

Mouse models of MCMV infection revealed that NK cells are not merely "killers" of infected targets, but very complex lymphocytes endowed with the molecular machinery necessary to perform a broad spectrum of functions. As part of the innate system, NK cells can immediately react to an infectious insult via fixed, germ-line encoded receptors that recognize pathogen-associated motifs. As the infection progresses, NK cells adapt, modulating the level of inflammatory mediators produced during the initial steps of infection. Finally, as adaptive responders, NK cells show clonal expansion, maintenance, and memory of previous insults in the case of reinfection.

Nevertheless, several questions remain: do NK cells recognize infections other than MCMV with their Ly49 repertoire of receptors? Can triggering of other NK receptors by MCMV-infected cells induce the same NK cell outcomes as in the Ly49H-m157 model (e.g., clonal expansion, memory, etc.)? What of other pathogens or different host species? Why does the host express so many different NK receptors targeting MCVM, several with opposite effects on NK cell activity (i.e., inhibitory versus activating)? Does the host benefit from the infection? If so, how? The possible paths of investigation are plentiful. As more inquiries are resolved, which is currently the case in the thriving field of NK cells in cancer, we will be able to harness the power of these lymphocytes in the treatment of infectious diseases [68, 69].

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References

[1] S. D. Burke, V. F. Barrette, J. Gravel et al., "Uterine NK cells, spiral artery modification and the regulation of blood pressure during mouse pregnancy," *American Journal of Reproductive Immunology*, vol. 63, no. 6, pp. 472–481, 2010.

[2] L. L. Lanier, "Evolutionary struggles between NK cells and viruses," *Nature Reviews Immunology*, vol. 8, no. 4, pp. 259–268, 2008.

[3] C. A. Biron, K. S. Byron, and J. L. Sullivan, "Severe herpesvirus infections in an adolescent without natural killer cells," *New England Journal of Medicine*, vol. 320, no. 26, pp. 1731–1735, 1989.

[4] R. Gazit, B. Z. Garty, Y. Monselise et al., "Expression of KIR2DL1 on the entire NK cell population: a possible novel immunodeficiency syndrome," *Blood*, vol. 103, no. 5, pp. 1965–1966, 2004.

[5] L. L. Lanier, "Up on the tightrope: natural killer cell activation and inhibition," *Nature Immunology*, vol. 9, no. 5, pp. 495–502, 2008.

[6] T. Crough and R. Khanna, "Immunobiology of human cytomegalovirus: from bench to bedside," *Clinical Microbiology Reviews*, vol. 22, pp. 76–98, 2009.

[7] V. J. Lisnić, A. Krmpotić, and S. Jonjić, "Modulation of natural killer cell activity by viruses," *Current Opinion in Microbiology*, vol. 13, pp. 530–539, 2010.

[8] M. G. Brown and A. A. Scalzo, "NK gene complex dynamics and selection for NK cell receptors," *Seminars in Immunology*, vol. 20, no. 6, pp. 361–368, 2008.

[9] R. Biassoni, "Human natural killer receptors, co-receptors, and their ligands," *Current Protocols in Immunology*, no. 84, pp. 14.10.1–14.10.40, 2009.

[10] P. Höglund and P. Brodin, "Current perspectives of natural killer cell education by MHC class i molecules," *Nature Reviews Immunology*, vol. 10, no. 10, pp. 724–734, 2010.

[11] K. M. Hsu, J. R. Pratt, W. J. Akers, S. I. Achilefu, and W. M. Yokoyama, "Murine cytomegalovirus displays selective infection of cells within hours after systemic administration," *Journal of General Virology*, vol. 90, no. 1, pp. 33–43, 2009.

[12] T. Sacher, J. Podlech, C. A. Mohr et al., "The major virus-producing cell type during murine cytomegalovirus infection,

the hepatocyte, is not the source of virus dissemination in the host," *Cell Host and Microbe*, vol. 3, no. 4, pp. 263–272, 2008.

[13] T. A. Banks, S. Rickert, C. A. Benedict et al., "A lymphotoxin-IFN-β axis essential for lymphocyte survival revealed during cytomegalovirus infection," *Journal of Immunology*, vol. 174, no. 11, pp. 7217–7225, 2005.

[14] K. Schneider, A. Loewendorf, C. De Trez et al., "Lymphotoxin-mediated crosstalk between B cells and splenic stroma promotes the initial type I interferon response to cytomegalovirus," *Cell Host and Microbe*, vol. 3, no. 2, pp. 67–76, 2008.

[15] K. Tabeta, P. Georgel, E. Janssen, X. Du, K. Hoebe et al., "Toll-like receptors 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, pp. 3516–3521, 2004.

[16] A. Krug, A. R. French, W. Barchet et al., "TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function," *Immunity*, vol. 21, no. 1, pp. 107–119, 2004.

[17] K. Tabeta, K. Hoebe, E. M. Janssen et al., "The Unc93b1 mutation 3d disrupts exogenous antigen presentation and signaling via Toll-like receptors 3, 7 and 9," *Nature Immunology*, vol. 7, no. 2, pp. 156–164, 2006.

[18] V. R. DeFilippis, D. Alvarado, T. Sali, S. Rothenburg, and K. Früh, "Human cytomegalovirus induces the interferon response via the DNA sensor ZBP1," *Journal of Virology*, vol. 84, no. 1, pp. 565–598, 2010.

[19] V. A. K. Rathinam, Z. Jiang, S. N. Waggoner et al., "The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses," *Nature Immunology*, vol. 11, no. 5, pp. 395–402, 2010.

[20] M. Swiecki, S. Gilfillan, W. Vermi, Y. Wang, and M. Colonna, "Plasmacytoid dendritic cell ablation impacts early interferon responses and antiviral NK and CD8+ T cell accrual," *Immunity*, vol. 33, no. 6, pp. 955–966, 2010.

[21] G. C. Pien, A. R. Satoskar, K. Takeda, S. Akira, and C. A. Biron, "Cutting edge: selective IL-18 requirements for induction of compartmental IFN-γ responses during viral infection," *Journal of Immunology*, vol. 165, no. 9, pp. 4787–4791, 2000.

[22] K. B. Nguyen, T. P. Salazar-Mather, M. Y. Dalod et al., "Coordinated and distinct roles for IFN-$\alpha\beta$, IL-12, and IL-15 regulation of NK cell responses to viral infection," *Journal of Immunology*, vol. 169, no. 8, pp. 4279–4287, 2002.

[23] D. M. Andrews, A. A. Scalzo, W. M. Yokoyama, M. J. Smyth, and M. A. Degli-Esposti, "Functional interactions between dendritic cells and NK cells during viral infection," *Nature Immunology*, vol. 4, no. 2, pp. 175–181, 2003.

[24] T. P. Salazar-Mather, J. S. Orange, and C. A. Biron, "Early murine cytomegalovirus (MCMV) infection induces liver natural killer (NK) cell inflammation and protection through macrophage inflammatory protein 1α (MIP-1α)-dependent pathways," *Journal of Experimental Medicine*, vol. 187, no. 1, pp. 1–14, 1998.

[25] N. Sumaria, S. L. H. Van Dommelen, C. E. Andoniou, M. J. Smyth, A. A. Scalzo, and M. A. Degli-Esposti, "The roles of interferon-γ and perforin in antiviral immunity in mice that differ in genetically determined NK-cell-mediated antiviral activity," *Immunology and Cell Biology*, vol. 87, no. 7, pp. 559–566, 2009.

[26] S. L. H. van Dommelen, N. Sumaria, R. D. Schreiber, A. A. Scalzo, M. J. Smyth, and M. A. Degli-Esposti, "Perforin and granzymes have distinct roles in defensive immunity and immunopathology," *Immunity*, vol. 25, no. 5, pp. 835–848, 2006.

[27] A. A. Scalzo, N. A. Fitzgerald, A. Simmons, A. B. La Vista, and G. R. Shellam, "Cmv-1, a genetic locus that controls murine cytomegalovirus replication in the spleen," *Journal of Experimental Medicine*, vol. 171, no. 5, pp. 1469–1483, 1990.

[28] M. Rodriguez, P. Sebastian, P. Clark, and M. G. Brown, "Cmv1-independent antiviral role of NK cells revealed in murine cytomegalovirus-infected New Zealand white mice," *Journal of Immunology*, vol. 173, no. 10, pp. 6312–6318, 2004.

[29] S. G. Adam, A. Caraux, N. Fodil-Cornu et al., "Cmv4, a new locus linked to the NK cell gene complex, controls innate resistance to cytomegalovirus in wild-derived mice," *Journal of Immunology*, vol. 176, no. 9, pp. 5478–5485, 2006.

[30] K. A. Daniels, G. Devora, W. C. Lai, C. L. O'Donnell, M. Bennett, and R. M. Welsh, "Murine cytomegalovirus is regulated by a discrete subset of natural killer cells reactive with monoclonal antibody to Ly49H," *Journal of Experimental Medicine*, vol. 194, no. 1, pp. 29–44, 2001.

[31] S. H. Lee, J. R. Webb, and S. M. Vidal, "Innate immunity to cytomegalovirus: the Cmv1 locus and its role in natural killer cell function," *Microbes and Infection*, vol. 4, no. 15, pp. 1491–1503, 2002.

[32] A. O. Dokun, S. Kim, H. R. C. Smith, H. S. P. Kang, D. T. Chu, and W. M. Yokoyama, "Specific and nonspecific NK cell activation during virus infection," *Nature Immunology*, vol. 2, no. 10, pp. 951–956, 2001.

[33] H. Arase, E. S. Mocarski, A. E. Campbell, A. B. Hill, and L. L. Lanier, "Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors," *Science*, vol. 296, no. 5571, pp. 1323–1326, 2002.

[34] H. R. C. Smith, J. W. Heusel, I. K. Mehta et al., "Recognition of a virus-encoded ligand by a natural killer cell activation receptor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 13, pp. 8826–8831, 2002.

[35] E. J. Adams, Z. S. Juo, R. T. Venook et al., "Structural elucidation of the m157 mouse cytomegalovirus ligand for Ly49 natural killer cell receptors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 24, pp. 10128–10133, 2007.

[36] I. Bubić, M. Wagner, A. Krmpotić et al., "Gain of virulence caused by loss of a gene in murine cytomegalovirus," *Journal of Virology*, vol. 78, no. 14, pp. 7536–7544, 2004.

[37] H. Sjölin, E. Tomasello, M. Mousavi-Jazi et al., "Pivotal role of KARAP/DAP12 adaptor molecule in the natural killer cell-mediated resistance to murine cytomegalovirus infection," *Journal of Experimental Medicine*, vol. 195, no. 7, pp. 825–834, 2002.

[38] T. P. Cheng, A. R. French, B. F. M. Plougastel et al., "Ly49h is necessary for genetic resistance to murine cytomegalovirus," *Immunogenetics*, vol. 60, no. 10, pp. 565–573, 2008.

[39] N. Fodil-Cornu, S. H. Lee, S. Belanger et al., "Ly49h-deficient C57BL/6 mice: a new mouse cytomegalovirus-susceptible model remains resistant to unrelated pathogens controlled by the NK gene complex," *Journal of Immunology*, vol. 181, no. 9, pp. 6394–6405, 2008.

[40] S. H. Lee, A. Zafer, Y. De Repentigny et al., "Transgenic expression of the activating natural killer receptor Ly49H confers resistance to cytomegalovirus in genetically susceptible mice," *Journal of Experimental Medicine*, vol. 197, no. 4, pp. 515–526, 2003.

[41] A. Kielczewska, H. S. Kim, L. L. Lanier, N. Dimasi, and S. M. Vidal, "Critical residues at the Ly49 natural killer receptor's

homodimer interface determine functional recognition of m157, a mouse cytomegalovirus MHC class I-like protein," *Journal of Immunology*, vol. 178, no. 1, pp. 369–377, 2007.

[42] A. H. Davis, N. V. Guseva, B. L. Ball, and J. W. Heusel, "Characterization of murine cytomegalovirus ml57 from infected cells and identification of critical residues mediating recognition by the NK cell receptor Ly49H," *Journal of Immunology*, vol. 181, no. 1, pp. 265–275, 2008.

[43] N. V. Guseva, C. A. Fullenkamp, P. W. Naumann et al., "Glycosylation contributes to variability in expression of murine cytomegalovirus m157 and enhances stability of interaction with the NK-cell receptor Ly49H," *European Journal of Immunology*, vol. 40, no. 9, pp. 2618–2631, 2010.

[44] D. W. McVicar, R. Winkler-Pickett, L. S. Taylor et al., "Aberrant DAP12 signaling in the 129 strain of mice: implications for the analysis of gene-targeted mice," *Journal of Immunology*, vol. 169, no. 4, pp. 1721–1728, 2002.

[45] L. L. Lanier, "NK cell recognition," *Annual Review of Immunology*, vol. 23, pp. 225–274, 2005.

[46] A. J. Corbett, J. D. Coudert, C. A. Forbes, and A. A. Scalzo, "Functional consequences of natural sequence variation of murine cytomegalovirus m157 for Ly49 receptor specificity and NK cell activation," *Journal of Immunology*, vol. 186, no. 3, pp. 1713–1722, 2011.

[47] V. Voigt, C. A. Forbes, J. N. Tonkin et al., "Murine cytomegalovirus m157 mutation and variation leads to immune evasion of natural killer cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 23, pp. 13483–13488, 2003.

[48] M.-P. Desrosiers, A. Kielczewska, J.-C. Loredo-Osti et al., "Epistasis between mouse Klra and major histocompatibility complex class I loci is associated with a new mechanism of natural killer cell-mediated innate resistance to cytomegalovirus infection," *Nature Genetics*, vol. 37, no. 6, pp. 593–599, 2005.

[49] A. Dighe, M. Rodriguez, P. Sabastian, X. Xie, M. McVoy, and M. G. Brown, "Requisite H2k role in NK cell-mediated resistance in acute murine cytomegalovirus-infected MA/My mice," *Journal of Immunology*, vol. 175, no. 10, pp. 6820–6828, 2005.

[50] X. Xie, A. Dighe, P. Clark, P. Sabastian, S. Buss, and M. G. Brown, "Deficient major histocompatibility complex-linked innate murine cytomegalovirus immunity in MA/My.L-H2 mice and viral downregulation of H-2 class I proteins," *Journal of Virology*, vol. 81, no. 1, pp. 229–236, 2007.

[51] A. Kielczewska, M. Pyzik, T. Sun et al., "Ly49P recognition of cytomegalovirus-infected cells expressing H2-D and CMV-encoded m04 correlates with the NK cell antiviral response," *Journal of Experimental Medicine*, vol. 206, no. 3, pp. 515–523, 2009.

[52] X. Xie, M. D. Stadnisky, and M. G. Brown, "MHC class I D locus and Ly49G2+ NK cells confer H-2 resistance to murine cytomegalovirus," *Journal of Immunology*, vol. 182, no. 11, pp. 7163–7171, 2009.

[53] X. Xie, M. D. Stadnisky, E. R. Coats et al., "MHC class I D expression in hematopoietic and nonhematopoietic cells confers natural killer cell resistance to murine cytomegalovirus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 19, pp. 8754–8759, 2010.

[54] M. T. Orr, W. J. Murphy, and L. L. Lanier, ""Unlicensed" natural killer cells dominate the response to cytomegalovirus infection," *Nature Immunology*, vol. 11, no. 4, pp. 321–327, 2010.

[55] M. Babić, M. Pyzik, B. Zafirova et al., "Cytomegalovirus immunoevasin reveals the physiological role of "missing self" recognition in natural killer cell dependent virus control in vivo," *Journal of Experimental Medicine*, vol. 207, no. 12, pp. 2663–2673, 2010.

[56] C. E. Andoniou, S. L. H. van Dommelen, V. Voigt et al., "Interaction between conventional dendritic cells and natural killer cells is integral to the activation of effective antiviral immunity," *Nature Immunology*, vol. 6, no. 10, pp. 1011–1019, 2005.

[57] S. H. Robbins, G. Bessou, A. Cornillon et al., "Natural killer cells promote early CD8 T cell responses against cytomegalovirus.," *PLoS Pathogens*, vol. 3, no. 8, article e123, 2007.

[58] S. H. Lee, S. Girard, D. Macina et al., "Susceptibility to mouse cytomegalovirus is associated with deletion of an activating natural killer cell receptor of the C-type lectin superfamily," *Nature Genetics*, vol. 28, no. 1, pp. 42–45, 2001.

[59] S. H. Lee, K. S. Kim, N. Fodil-Cornu, S. M. Vidal, and C. A. Biron, "Activating receptors promote NK cell expansion for maintenance, IL-10 production, and CD8 T cell regulation during viral infection," *Journal of Experimental Medicine*, vol. 206, no. 10, pp. 2235–2251, 2009.

[60] V. Bekiaris, O. Timoshenko, T. Z. Hou et al., "Ly49H+ NK cells migrate to and protect splenic white pulp stroma from murine cytomegalovirus infection," *Journal of Immunology*, vol. 180, no. 10, pp. 6768–6776, 2008.

[61] D. M. Andrews, M. J. Estcourt, C. E. Andoniou et al., "Innate immunity defines the capacity of antiviral T cells to limit persistent infection," *Journal of Experimental Medicine*, vol. 207, no. 6, pp. 1333–1343, 2010.

[62] C. A. Benedict, A. Loewendorf, Z. Garcia, B. R. Blazar, and E. M. Janssen, "Dendritic cell programming by cytomegalovirus stunts naive T cell responses via the PD-L1/PD-1 pathway," *Journal of Immunology*, vol. 180, no. 7, pp. 4836–4847, 2008.

[63] J. G. O'Leary, M. Goodarzi, D. L. Drayton, and U. H. von Andrian, "T cell- and B cell-independent adaptive immunity mediated by natural killer cells," *Nature Immunology*, vol. 7, no. 5, pp. 507–516, 2006.

[64] J. C. Sun, J. N. Beilke, and L. L. Lanier, "Adaptive immune features of natural killer cells," *Nature*, vol. 457, no. 7229, pp. 557–561, 2009.

[65] A. M. Jamieson, P. Isnard, J. R. Dorfman, M. C. Coles, and D. H. Raulet, "Turnover and proliferation of NK cells in steady state and lymphopenic conditions," *Journal of Immunology*, vol. 172, no. 2, pp. 864–870, 2004.

[66] M. A. Cooper, J. M. Elliott, P. A. Keyel, L. Yang, J. A. Carrero, and W. M. Yokoyama, "Cytokine-induced memory-like natural killer cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 6, pp. 1915–1919, 2009.

[67] S. Paust, H. S. Gill, B.-Z. Wang et al., "Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses," *Nature Immunology*, vol. 11, no. 12, pp. 1127–1135, 2010.

[68] M. Terme, E. Ullrich, N. F. Delahaye, N. Chaput, and L. Zitvogel, "Natural killer cell-directed therapies: moving from unexpected results to successful strategies," *Nature Immunology*, vol. 9, no. 5, pp. 486–494, 2008.

[69] S. K. Lee and S. Gasser, "The role of natural killer cells in cancer therapy," *Frontiers in Bioscience*, vol. 2, pp. 380–391, 2010.

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Review Article

Involvement of Activating NK Cell Receptors and Their Modulation in Pathogen Immunity

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Natural Killer (NK) cells are endowed with cell-structure-sensing receptors providing inhibitory protection from self-destruction (inhibitory NK receptors, iNKRs, including killer inhibitory receptors and other molecules) and rapid triggering potential leading to functional cell activation by Toll-like receptors (TLRs), cytokine receptors, and activating NK cell receptors including natural cytotoxicity receptors (NCRs, i.e., NKp46, NKp46, and NKp44). NCR and NKG2D recognize ligands on infected cells which may be endogenous or may directly bind to some structures derived from invading pathogens. In this paper, we address the known direct or indirect interactions between activating receptors and pathogens and their expression during chronic HIV and HCV infections.

1. Introduction

Natural Killer (NK) cells represent a highly specialized lymphoid population initially identified by a potent cytolytic activity against tumor or virus infected cells. Different from T or B lymphocytes, they do not express clonally distributed receptors for antigen [1, 2], while their function is finely regulated by a balance of inhibitory and activating receptors. NK cell inhibitory receptors, recognizing mostly HLA class I molecules on "self" cells (notable exceptions to this concept are represented, among others, by Siglec7 and IRP60 recognizing non-HLA-related structures), turn NK cells "off" and represent the major failsafe device to prevent NK-mediated attack of normal HLA class I^+ autologous cells. On the other hand, the "on" signal is delivered when NK cells interact with target cells that lack MHC class I molecules and at the same time are triggered through activating molecules expressed by these cells (Figure 1). Activating stimuli may be delivered to NK cells through triggering via Toll-like receptors (TLRs) including TLR2, TLR3, TLR7/8, TLR9, interleukin receptors (IL-2, IL-12, IL-15, IL-18), and combinations thereof (e.g., IL-2 + IL-15, IL-2 + IL12, IL-12 + IL-18), or activatory receptors representing an array of different molecules expressed on their surface including natural cytotoxicity receptors (NCRs), NKG2D, NKG2C (a lectin-type triggering receptor which dimerizes with CD94), 2B4 (CD244), NKp80, DNAM-1, NTB-A, and the receptor for IgFc (CD16) [3].

The receptors responsible for NK cell activation in the process of natural cytotoxicity are collectively termed natural cytoxicity receptors (NCRs): NKp46 [4, 5], NKp44 [6, 7], and NKp30 [8]. Their expression is mostly restricted to NK cells, and particularly in the case of NKp46, they represent the most accurate surface markers for human NK cell identification. Exceptions for NK cell identification have been documented. NKp44 may be detected on the surface of a minority of peripheral plasmacytoid dendritic cells [9] but on a relevant fraction of tissue-resident pDC [10] and NKp30 may be expressed by umbilical cord T-lymphocytes upon activation [11]. While NKp30 and NKp46 enable a precise identification of NK cells, regardless of whether these cells are resting or activated, NKp44 is selectively expressed only by activated NK cells [6, 7, 12] and should be differentiated from

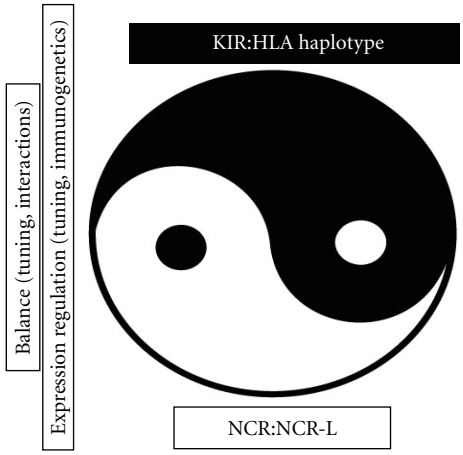

Figure 1: Diagram resuming the balance of activating and inhibitory NK cell receptor:ligand relationships.

NKp44 which is constitutively expressed on pDCs in tissues [10].

NCRs play a major role in NK-mediated killing of most tumor cell lines, as revealed by monoclonal antibody-mediated receptor-masking experiments [3, 12]. Moreover, their surface density on NK cells correlates with the magnitude of cytolytic activity against NK-susceptible target cells [13]. The ligands recognized by NCRs are still incompletely molecularly defined and may have variable expression on different cells [14]. However, as revealed by cytolytic assays, they are expressed by cells belonging to different histotypes [2, 15–17] and, in some cases, may be associated to neoplastic cells (e.g., B7-H6) [18] or to RNA viruses including influenza, dengue, or West Nile virus [19, 20]. NKG2D is another major NK-cell-triggering receptor belonging to the NKG2 family (type II membrane proteins characterized by a lectin-like domain) [21–23]. Contrary to the NCR, NKG2D is not restricted to NK cells but may be also expressed by cytolytic T lymphocytes [24]. NKG2D is specific for stress-inducible polymorphic MHC-class-I-related chain (MIC), MIC-A and MIC-B or ULBP proteins [25], which may be expressed upon cell infection [26] or transformation [3].

Other triggering surface molecules expressed by NK cells are shared by other leucocyte types and appear to function primarily as coreceptors. They may function to amplify signaling by true receptors. Two such coreceptors, 2B4 [27] and NTB-A [28], appear to serve a dual and opposite function, depending of availability of downstream regulating elements on their signaling pathways.

A triggering surface molecule termed NKp80 has been identified by the generation of specific mAb (MA152 and LAP171) [29]. NKp80 is expressed by virtually all fresh NK cells derived from peripheral blood as well as by a minor T-cell subset characterized by the $CD3^{+}CD56^{+}$ surface phenotype [30] and binds to AICL on target cells [31]. NKp80 has so far no specific reactivity with pathogen-associated structures.

Another molecule behaving as triggering coreceptor in NK cells was described following attempts to identify the cellular ligands of triggering receptors [32]. DNAM-1 is a transmembrane protein involved in lymphocyte adhesion and signaling. In addition to NK cells, it is expressed also on T cells, monocytes, and a small subset of B lymphocytes. The role of DNAM-1 in NK-mediated killing varies with the different target cells analyzed thus far, suggesting differences in the expression of DNAM-1 ligands. Indeed, carcinomas and hematopoietic cell lines express PVR and Nectin-2, and their lysis involves DNAM-1. On the other hand, most EBV-transformed B cell lines analyzed do not express PVR nor Nectin-2, and their lysis does not involve DNAM-1 [32]. Thus, PVR and Nectin-2 represent the major (if not only) ligands of DNAM-1. As in the case of the other triggering receptors, the NK cell activation via DNAM-1 is controlled by HLA-class-I-specific inhibitory receptors. As a consequence, normal cells are usually protected from lysis.

Other triggering receptors including CD27, CRTAM, CD96, CD100(SEMA4D), PSGL1, and CD319 (SLAMF7) may be expressed by NK cells and have known ligands [3]. None of them has been so far reported to significantly interact with invading pathogens.

2. NCR-Pathogen Interactions

NK cells are a central component of innate immune response, comprising the first line of defense against a variety of tumors and microbial pathogens, including viruses, bacterial, fungal, and other intracellular parasites [33–36]. The lytic activity of NK cells is controlled by complex interactions of inhibitory and activating receptors with specialized signalling machinery, and at times, the possibility to directly detect pathogen-derived molecules, independent of Toll-like receptors [2, 37, 38].

In addition to possible direct activation of NK cells via TLR by pathogen-derived molecular structures (PAMP, e.g., LPS, RNA, DNA), accumulating evidences over recent years have linked NCRs on NK cells with direct or indirect recognition of pathogen-associated structures. Given their role for sensing intracellular pathogen-infected cells, under particular conditions, these observations may bear relevant importance in the outcome of an immune response.

One should also consider that, even in the presence of weak inhibitory activity by KIR or other inhibitory receptors (e.g., KIR3DL2-HLA-C1 homozygosity), defects in NCR expression could lead to failure in recognizing pathogen-associated molecules acting as NCR ligands on infected cells, leading to variable degrees of derangement in NK cell function (Figure 2). The balance of KIR carriage:HLA class I expression and NKG2A/CD94:HLA-E or CD85j(LIR1/ILT2):/HLA class I expression needs also to be considered in these cases. In other terms, although KIR:HLA carriage is known to influence NK cell activation [39], this pathway alone is not exhaustively representing overall NK cell function, for example in KIR-negative $CD56^{bright}$ NK cells, in NK cell precursors (which are KIR-NKG2A+ and KIR-NKG2A−, resp.), or in CD56dull NK cells carrying KIR2DL3 on an HLA-C1 homozygous background but in addition express high levels of CD85J (LIR1/ILT2).

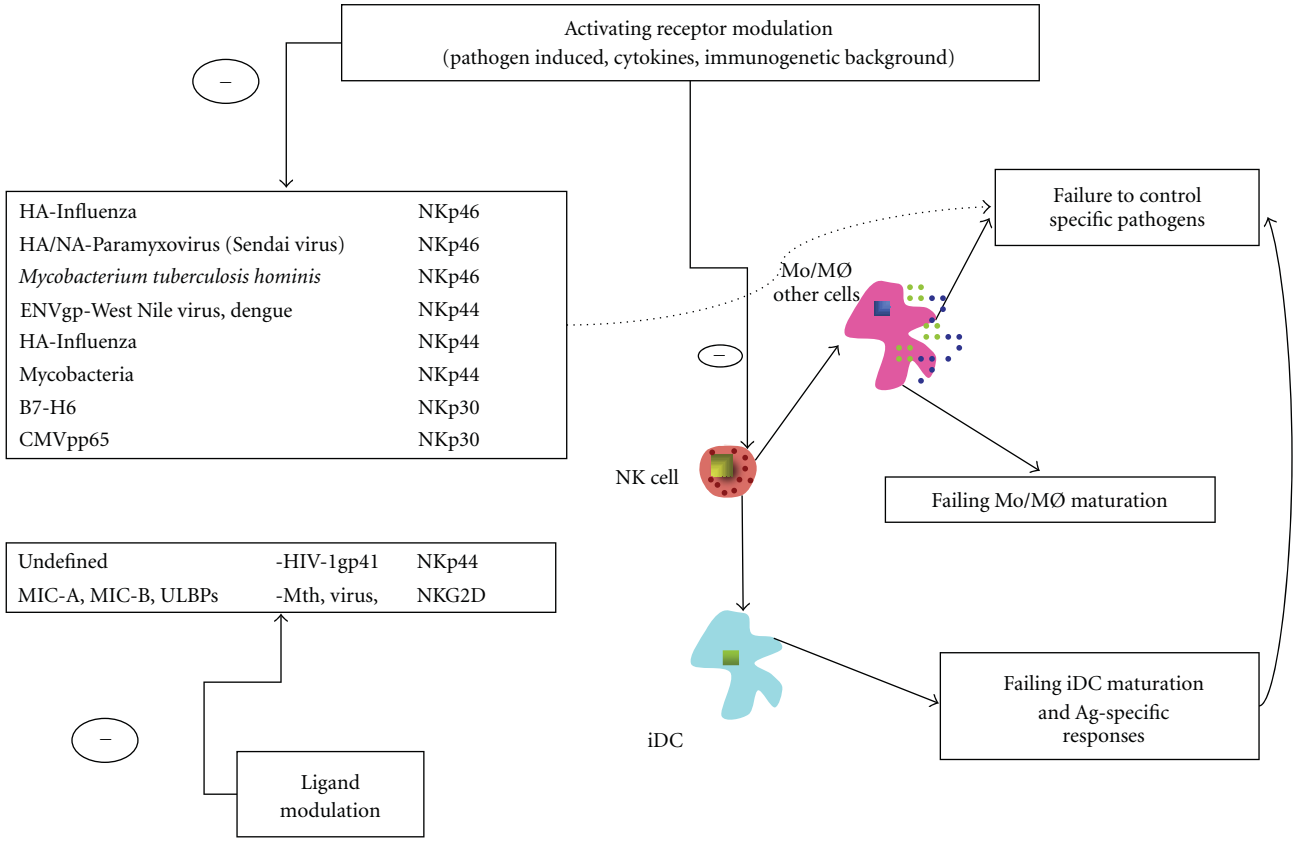

Figure 2: Diagram representing possible consequences of activating receptor modulation, alone, on the control of acute or chronic infections.

NCRs have been found to interact with infected cells through recognition of virus-encoded molecules. Sendai virus (SV) haemagglutinin-neuraminidase (HN) and Influenza virus (IV) haemagglutinin (HA) both recognize terminal N-acetylneuraminic acid residues (sialic acids) attached to Gal. IV HA and SV HN, bind to sialic acid residues on NKp46 and NKp44 [19, 40], enabling NCR-mediated lysis of IV or SV-infected cells. Subsequent clinical observations corroborated these findings and showed that lethal IV infection in humans may be associated to absence of NKp46 on NK cells [41]. NKp46 is also involved in the detection and lysis of vaccinia-virus-infected cells, together with NKp30 and NKp44 [42] although viral ligands have not been characterized.

NKp44 has been reported to interact directly to cell-free mycobacteria or other bacteria [43] and to play a role in the recognition of virus-infected cells. In fact, following the original description of a putative interaction of NKp46 with influenza virus haemagglutinin and Sendai Virus hemagglutinin-neuraminidase [19], also NKp44 was found to interact with these proteins [40]. Direct interaction of NKp44 with virus-infected cells and with virus or virus-like particles, has been recently shown also with regard to the envelope protein of flavivirus (West Nile virus, dengue Virus) [20]. This leaves open the question of the potential relationship of HCV (another member of the *Flaviviridae* family) and NCR expression with regard to disease course. NKp44 has been involved in disease pathogenesis also with regard to HIV. HIV-1-infected $CD4^+$ cells are reported to be targeted by NK cells via a NKp44L that is induced by an HIVgp41 peptide [44]. The presence of antibodies to this peptide *in vivo* would prevent NK cell recognition and disposal of infected cells. In HIV-infected patients, these antibodies are correlated with $CD4^+$ cell numbers and NKp44L expression on $CD4^+$ cells [45]. There has been so far no nearer characterization of this ligand, although it has been recently shown that the 3S motif of gp41 binds to gC1qR (a receptor for the globular domain of C1q) on $CD4^+$ T cells and induces activation of a signalling cascade that leads to NKp44L expression also on uninfected $CD4^+$ T cells [46]. Thus, NKp44 may be involved not only in protection from overwhelming infection by direct interaction with cells infected by viruses belonging to different virus families (*Orthomyxo-, Paramyxo-, Flavi-, and Lentiviridae*) but may also play a role in the pathogenesis of hyperactivation syndromes (as with influenza or dengue) or in immune depletion (e.g., HIV-1).

Several aspects remain be further clarified in this area, as direct NK-pathogen interaction via an activating receptor that is only expressed upon NK cell activation (NKp44), entails recognition only after full NK cell activation and not upon initial events following virus entry.

In line with what has been recently shown for NKp44, also NKG2D interacts with multiple cellular- or pathogen-derived ligands that trigger NK cell cytotoxicity. NKG2D

binds to the polymorphic MHC-class-I-related chain (MIC), MIC-A and MIC-B [47], and also binds to UL-16-binding proteins (ULBPs), which are MHC class-I-like molecules that are expressed by tumor cell lines. ULBPs enhance the capacity of NK cells to lyse tumors and to produce cytokines such as IFNγ and TNFα [48, 49] and are upregulated during heat shock or during infection with CMV [50, 51].

Opposite to NKp44 and NKp46, there is little evidence for direct interaction of NKG2D with pathogen(s); however, its ligands may be either induced or downmodulated upon infection by several pathogens. In the case of rodent poxviruses such as ectromelia virus (mousepox virus), NKG2D ligands are upregulated on infected cells [52, 53], while it appears that human poxviruses such as vaccinia virus do not affect NKG2D expression in their host but rather affect NKp46, NKp44, and NKp30 through IFN-α-mediated increased expression [42, 54]. NKG2D appears to play a relevant role in killing HIV-1-infected $CD4^+$ cells, as its ligands are still expressed in infected cells *in vitro* [26]. However, HIV Nef is specifically downmodulating ULBP2 [55], thus, preventing NKG2D-mediated cytotoxicity. Downregulation of NKG2D ligands MIC-A and MIC-B is also induced by the *K5* immune evasion gene of KSHV (HHV-8). The same gene product also determines downregulation of the ligand of NKp80 (i.e., AICL), thus, simultaneously maiming both NK- and T-cell responses against HHV-8 at multiple levels [56]. Human CMV has developed a similar protective system against both NKG2D-expressing T cells and NK cells, as its UL16 protein binds to 3 or the main NHG2D ligands (i.e., MICB, ULBP1, ULBP2), thus, preventing them from being expressed by infected cells, thereby, avoiding cell killing by NKG2D-expressing NK cells [57]. A less extensive NKG2D-Ligand targeting (i.e., MICA alone) has been reported also for HCV via its NS3/4A serine protease [58]. Overall, there are still conflicting views in this area that could need additional attention to improve our understanding of the role played by NKG2D and NKG2D-interference during infection by intracellular pathogens comparing human and animal models. Since NKG2D is also expressed by a relevant proportion of $CD8^+$ CTLs, additional aspects need do be addressed comprehensively to improve our understanding of the relative role of NK cells and CD8 CTLs in disease pathogenesis to help advise possible interventions.

There are few if any reports suggesting a direct interaction of other triggering coreceptors 2B4 (CD224) or DNAM-1 or their ligands (i.e., CD48 and poliovirus receptor (CD155) and Nectin-2 (CD112) with viral products) [32, 59, 60]. One possible exception is represented by NKp80, as mentioned above, which is targeted by HHV-8 proteins which induce downmodulation of its ligand in infected cells.

3. NCR Involvement in the Pathogenesis of NK Cell Dysfunction During HIV Infection

The strongest evidence for a role of the immune system in controlling HIV-1 disease comes from a number of epidemiological studies demonstrating a strong influence of individual HLA class I alleles on determining the rate of HIV-1 disease progression [61]. Several subsets of cells belonging to the hematopoietic lineage express receptors that bind to HLA class I molecules, including $CD8^+$ T cells, monocytes, dendritic cells, and NK cells.

A number of observations strongly suggest that $CD8^+$ T cells play an important role in the containment of HIV infection. For example, exposed-uninfected seronegative subjects lack specific humoral responses to HIV-1 but have detectable $CD8^+$CTL responses and mucosal IgA immunity [62–65]. However, HIV-1-specific $CD8^+$ T-cell immunity alone is not sufficient to explain the large heterogeneity observed in the clinical manifestation of HIV-1 disease. More recent advances in the understanding of the immune response to viral infections support the involvement of additional components of the innate immune system in the control of HIV-1 disease and might help to identify the mechanism underlying protective immunity in HIV-1 infection.

HIV-1 infection is associated with significant changes in NK cell subset distributions and function in the peripheral circulation which were detected already at the beginning of the epidemic and were subsequently systematically evaluated and reviewed [66–68]. Several reports have shown a dramatic reduction in the proportion of $CD3^-$ $CD56^+$ NK cells [69, 70], and particularly $CD56^{bright}$ $CD16^{+/-}$ [71]. This reduction appears to be partially attributable to the emergence of a novel subset of NK cells that is rare in healthy individuals, $CD3^-$ $CD56^-$ $CD16^+$ NK cells [68, 72]. Earlier work showed that NK cells in HIV-viremic patients displayed a functionally relevant and dramatic reduction in NCRs [67], which is accompanied by relevant activation, as determined by HLA-DR and CD69 expression [73]. A consistent fraction of these cells fall also in the subsequently identified $CD56^-$ $CD16^+$ $CD3^-$ "exausted" NK cell subset [72]. Since this subset becomes prominent in individuals with active viral replication at the expense of the two other subsets of cells (i.e., $CD56^{dim}$ and $CD56^{bright}$ NK cells), it needs to be accounted for to evaluate the overall number of NK cells in patients with HIV-1 infection. NK cells with defective NCR expression in HIV patients, among these also $CD3^-$ $CD56^+$ $CD16^+$ NK cells, have strongly reduced NK cell effector functions, including killing, cytokine secretion, and antibody-dependent cellular cytotoxicity (ADCC), and exhibit aberrant DC-editing activity [67, 73, 74]. DC-editing by NK cells may take place whenever DCs and NK cells interact in secondary lymphoid organs. DCs that do not express functionally relevant HLA class I molecule densities are subject to NK cell killing (e.g., iDCs and incompletely mature DCs). NK cells in turn induce DC maturation through IFN-γ and TNF-α production [75–79]. Since incompletely mature DCs produce higher levels of IL-10 and lower quantities of IL-12 and show less efficient antigen presentation [80], the efficiency of NK-DC interactions "crosstalk" including cytokine production and NKp30/DNAM-1 expression may variably shape subsequent downstream T-cell and B-cell adaptive responses.

In addition to changes in NK subpopulations associated with HIV infection, there are also marked changes in NK surface receptor expression that are related to loss of function. With HIV viremia, there is an overall decrease in surface receptor density of NKp46 and NKp30 found on freshly

drawn NK cells and dysfunction in NKp44 *de novo* expression upon stimulation *in vitro* resulting in what has been termed an NCR^{dull} phenotype [67]. In the $CD56^{dim}$ subset, the proportion of cells expressing NKp46 and NKp30 and their cytolytic activity decrease with disease progression [81]. Moreover, a concomitant increase of KIR density is also observed not only on $CD8^+$ CTL, but also on NK cells, thus, setting the basis for an increased inhibitory potential of cytolytic cells [82–84]. The downmodulation of activating receptors and upregulation of KIRs result in measurable functional defects in NK-cell-mediated cytotoxicity regardless of the NK cell subpopulation.

The overall cause of NK cell dysfunction during HIV infection is poorly understood. The induction of NKp44L by HIVgp41 on infected and uninfected $CD4^+$ cells and the as yet poorly characterized downmodulation of activatory receptor and coreceptor ligands are likely to be involved [26, 46]. Despite isolated reports on the possibility of infection of $CD4^-$ expressing or activated NK cells [85, 86], peripheral NK cells are not infected by HIV [72]. This phenomenon may be rather attributed to cells different from NK cells [87] and is currently not regarded as a major mechanism leading to NK cell dysfunction.

Antiretroviral treatment (ART) does not significantly affect NK cell function recovery, as IFNγ production [88], and NCR expression may be persistently impaired even after successful ART and virus control in patients with $CD4^+$ cell numbers >500/μL [89]. Variable NCR expression during ART, thus, appears to contribute to clinical disease course upon treatment interruption. Thus, differences in innate immune balance during ART may be associated to differential control of HIV. Their understanding could explain clinical differences in individual patients that are not reflected by $CD4^+$ cell counts alone.

Overall, therefore, although KIR:HLA carriage may significantly affect the course of HIV infection [61, 90, 91], direct or indirect disruption of NCR/NCR-ligand expression by HIV represents a significant event that needs to be considered in parallel when evaluating NK cell activating/inhibiting balance. Disruption of NK-DC interaction and DC editing by NK cells via NKp30 [76, 78, 79] and DNAM-1 [77] is likely to lead to inefficient selection of DC maturation with inefficient downstream antigen-specific T- and B-cell responses [74, 92].

4. NCRs in Nonhuman Primates and HIV/SIV Infection

Studies describing NKp46, NKp30, NKG2D, and NKp80 in animal models of HIV infection revealed that NCR expression in SHIV-infected macaques does not reflect the downmodulation observed in HIV-infected humans [93, 94]. Importantly, defective transcription of NKp44 has been observed in macaques and this explains why no surface expression of this NCR is detected in macaques (*Macaca mulatta and Macaca fascicularis*) [93–95], thus, raising questions on the interpretation of some experiments in this animal model with regard to NK cell regulation.

On the contrary, although NKp44 transcription and induction appears to be differently regulated in chimpanzees [95] compared to humans, this primate species has a full set of NCRs [96]. A relevant difference with *Homo sapiens* relies in NKp30 expression and regulation and may impact on the relative resistance to progressive HIV infection in this species. Indeed, in both uninfected and HIV-infected chimpanzees NKp30 is not- or poorly expressed but is *de novo* expressed at levels comparable to human NK cells upon cell activation [96]. Thus, even during HIV replication, NK:DC crosstalk via NKp30 is likely to be dampened and may explain the low level of immune activation even in the presence of active replication of a virus that in humans thrives on cell activation.

5. NCRs and NK Cell Function in HCV Infection

NK cells have been implicated in all stages of HCV infection in both genetic and functional studies. This role may be either direct, by targeting hepatocytes, or indirect by influencing other key immunocytes such as DCs or T cells. NK cells comprise 5–20% of peripheral blood mononuclear cells but make up a substantially greater proportion (30–50%) of lymphocytes in the liver [97]. Intrahepatic NK cells in murine models may behave differently (i.e., hyporesponsiveness) compared to NK cells in other districts, due to a presumed "tolerogenic" environment in the liver. They are less cytotoxic and have an altered cytokine profile producing lower level of IFNγ and greater levels of Immunomodulatory cytokines, such as IL-10, compared to peripheral blood and splenic NK cells [98].

Human peripheral blood NK cells are involved in the acute phase of HCV infection, with an increase in $CD56^{bright}$ NK cells and associated reduction in $CD56^{dim}$ subset [99]. A decline in the $CD56^{bright}$ population is observed in patients spontaneously clearing the virus and reaching levels comparable to healthy control individuals within 1–3 months. This return to baseline is not observed in those that proceed to chronic HCV infection. In the acute phase of infection, expression of NKG2D is increased with augmented IFNγ production and cytotoxicity [99]. In addition, peak NK cell activation and degranulation precedes or coincides with peak T-cell responses, and a correlation was observed between NK cell degranulation and the magnitude of HCV-specific T-cell responses [100]. Thus, recent evidences agree with a direct involvement of NK cell responses during acute HCV infection favoring induction and priming of downstream T-cell responses leading to virus clearance.

During chronic HCV infection, peripheral blood NK cell frequency (both absolute number and percentage of total lymphocytes) is reduced in chronic HCV compared with healthy individuals [101–104]. NK cell frequency increases following successful antiviral therapy [105]. A reduction in peripheral blood NK cell frequency in patients with chronic HCV as compared to spontaneous resolvers has also been noted [106, 107]. These observations may be at least in part explained by a significant reduction of IL-15 (a pivotal cytokine for NK cell development, proliferation,

and function) concentrations/levels which is observed in HCV patients as compared to healthy controls [103].

Several studies in chronic HCV patients have documented a relative increase in circulating $CD56^{bright}$ (but not $CD56^{dim}$ NK cells) compared to healthy individuals and spontaneous resolvers [102, 106, 108]. Bonorino et al. demonstrated that the relative proportions of $CD56^{dim}$ and $CD56^{bright}$ NK cells in the liver are altered in chronic HCV observing that 80.5% of intrahepatic NK cells were $CD56^{dim}$ as compared to 94% in peripheral blood, and 19.5% intrahepatic NK cells were $CD56^{bright}$ as compared to 6.0% in peripheral blood [102]. This implies that the decreased frequency of $CD56^{dim}$ in the periphery is not related to their sequestration in the liver, although there are no nearer data on the relative proportion of $CD56^{dim}$ and $CD56^{bright}$ NK cells in the healthy liver. Changes in NK cell phenotype may not necessarily reflect changes in subset distribution. $CD56^{bright}$ NK cells are KIR-negative and NKG2A-positive, and one of the most consistent findings so far has been an increase in NKG2A expression in chronic HCV infection [108–110]. This occurs on both intrahepatic and peripheral blood NK populations [102] and also involves $CD56^{dim}$ NK cells.

Contrary to HIV infection, there has been so far less consensus on NCR expression during chronic HCV infection. Increased proportions and density of NCRs, including NKG2C, NKp44, NKp30, and NKp46 have been reported [108, 111, 112]. Initial reports of decreased expression of NKp46 [113] have not been subsequently confirmed. Similarly, there is conflicting evidence with respect to NKG2D expression which has been reported as being upregulated, downregulated, and also unchanged during chronic HCV infection [108, 109, 112]. However, these apparently opposite findings could be reconciled by different treatment responses in different cohorts [114].

Thus, the interaction of HCV with NCRs on NK cells is less direct compared to HIV, as direct virus challenge does not impair NK cell function [115], and may be mediated by other as yet poorly understood viral factors [116] or by possible immunogenetic traits that would parallel those observed for the other side of the (NK cell) coin (i.e., KIR:HLA interactions) [117].

6. NCRs during *Mycobacterium tuberculosis hominis* Infection

Different sets of observations support the notion that NK cells may be involved in the control of mycobacterial infections, with particular emphasis on *Mycobacterium tuberculosis hominis* (Mth). First, NK cells from healthy donors (HDs) can directly respond to products via TLR-2 [118]. Moreover, NK cells respond to mycobacterium-infected mDC [75]. Direct interaction between NCRs and Mth has been suggested (see above). A direct link between NK cells and Mth-infected cells has been shown to be mediated by the interaction of NKp46- and NKG2D-activating receptors on NK cells with infected monocytes [119]. Evidences indicating a relevant role for NK-mediated control of Mth replication are provided by experiments using cells from HDs showing their promotion of intracellular killing of mycobacteria [120, 121] and by their ability to lyse monocytes infected with Mth, *Mycobacterium tuberculosis bovis* Bacille Calmette-Guerin or *Mycobacterium avium intracellulare* [122]. In addition, the recent description of reduced mDC and pDC numbers and function in patients with TB [123] suggests that this, in turn, may impair NK cell function (e.g., through impaired production of cytokines promoting NK cell activation such as IL-12 and IL-15) contributing to reduce lysis of Mth-infected macrophages which are, in turn, subjected to the activation of antiapoptotic pathways by Mth [119, 124].

NK cells have been shown to play a fundamental role in the maintenance of efficient Ag-specific $CD8^+$ CTL responses in healthy humans with latent Mth infection [125]. In line with these observations, patients with recent onset of pulmonary TB have low-level expression of both NKp46 and NKp30 [126], suggesting that NK-cell-driven downstream adaptive responses may also be defective. In addition, patients successfully recovering from pulmonary TB with standard treatment (rifampicin, isoniazid, ethambutol, and pyrazinamide for 2 months followed by 24 months of rifampicin and isoniazid) do recover IFNγ production by NK cells but fail to recover NKp30 and NKp46 expression [126] and in some cases do so even after 3–5 years from end of treatment (A. De Maria, personal observation). These observations could be explained by the hypothetical scenario of immunogenetically determined regulation on NCR expression that may influence different interindividual susceptibility to latent Mth reactivation with onset of disease.

7. Concluding Remarks

So far, the role of NCRs in the interaction of pathogens with NK cells has been largely considered ancillary to cytokine- or TLR-mediated activation and to KIR:HLA modulation of NK cell function. Over the last 10 years accumulating evidences point to the possibility of viruses to directly interact with NCRs to tamper with their expression and to interfere with NCR ligand expression on target cells, thus, indirectly reducing NK cell function irrespective of the KIR : HLA haplotype (Figure 2).

Improving our knowledge of the mechanism(s) and of the regulation of NCR expression and function and integrating this information with inhibitory receptor expression and function will improve the prospective management of patients with potentially lethal or invalidating acute infections including influenza, dengue, or HCV and optimize treatment strategies of chronic invalidating persistent infections such as HIV, TB, and HCV.

References

[1] G. Trinchieri, "Biology of natural killer cells," *Advances in Immunology*, vol. 47, pp. 187–376, 1989.

[2] L. Moretta, R. Biassoni, C. Bottino, M. C. Mingari, and A. Moretta, "Human NK-cell receptors," *Immunology Today*, vol. 21, no. 9, pp. 420–422, 2000.

[3] E. Vivier, D. H. Raulet, and A. Moretta, "Innate or adaptive immunity? The example of natural killer cells," *Science*, vol. 331, no. 6013, pp. 44–49, 2011.

[4] S. Sivori, M. Vitale, L. Morelli et al., "p46, a novel natural killer cell-specific surface molecule that mediates cell activation," *Journal of Experimental Medicine*, vol. 186, no. 7, pp. 1129–1136, 1997.

[5] A. Pessino, S. Sivori, C. Bottino et al., "Molecular cloning of NKp46: A novel member of the immunoglobulin superfamily involved in triggering of natural cytotoxicity," *Journal of Experimental Medicine*, vol. 188, no. 5, pp. 953–960, 1998.

[6] M. Vitale, C. Bottino, S. Sivori et al., "NKp44, a novel triggering surface molecule specifically expressed by activated natural killer cells, is involved in non-major histocompatibility complex-restricted tumor cell lysis," *Journal of Experimental Medicine*, vol. 187, no. 12, pp. 2065–2072, 1998.

[7] C. Cantoni, C. Bottino, M. Vitale et al., "NKp44, a triggering receptor involved in tumor cell lysis by activated human natural killer cells, is a novel member of the immunoglobulin superfamily," *Journal of Experimental Medicine*, vol. 189, no. 5, pp. 787–795, 1999.

[8] D. Pende, S. Parolini, A. Pessino et al., "Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells," *Journal of Experimental Medicine*, vol. 190, no. 10, pp. 1505–1516, 1999.

[9] A. Fuchs, M. Cella, T. Kondo, and M. Colonna, "Paradoxic inhibition of human natural interferon-producing cells by the activating receptor NKp44," *Blood*, vol. 106, no. 6, pp. 2076–2082, 2005.

[10] I. Bonaccorsi, C. Cantoni, P. Carrega et al., "The immune inhibitory receptor LAIR-1 is highly expressed by plasmacytoid dendritic cells and acts complementary with NKp44 to control IFNα production," *PLoS ONE*, vol. 5, no. 11, Article ID e15080, 2010.

[11] Q. Tang, B. Grzywacz, H. Wang et al., "Umbilical cord blood T cells express multiple natural cytotoxicity receptors after IL-15 stimulation, but only NKp30 is functional," *Journal of Immunology*, vol. 181, no. 7, pp. 4507–4515, 2008.

[12] R. Biassoni, C. Cantoni, D. Pende et al., "Human natural killer cell receptors and co-receptors," *Immunological Reviews*, vol. 181, pp. 203–214, 2001.

[13] S. Sivori, S. Parolini, M. Falco et al., "2B4 functions as a co-receptor in human NK cell activation," *European Journal of Immunology*, vol. 30, no. 3, pp. 787–793, 2000.

[14] A. Byrd, S. C. Hoffman, M. Jarahian, F. Momburg, and C. Watzl, "Expression analysis of the ligands for the natural killer cell receptors NKp30 and NKp44," *PLoS ONE*, vol. 2, no. 12, Article ID e1339, 2007.

[15] S. Sivori, S. Parolini, E. Marcenaro et al., "Involvement of natural cytotoxicity receptors in human natural killer cell-mediated lysis of neuroblastoma and glioblastoma cell lines," *Journal of Neuroimmunology*, vol. 107, no. 2, pp. 220–225, 2000.

[16] R. T. Costello, S. Sivori, E. Marcenaro et al., "Defective expression and function of natural killer cell-triggering receptors in patients with acute myeloid leukemia," *Blood*, vol. 99, no. 10, pp. 3661–3667, 2002.

[17] D. Pende, P. Rivera, S. Marcenaro et al., "Major histocompatibility complex class I-related chain A and UL16-binding protein expression on tumor cell lines of different histotypes," *Cancer Research*, vol. 62, no. 21, pp. 6178–6186, 2002.

[18] C. S. Brandt, M. Baratin, E. C. Yi et al., "The B7 family member B7-H6 is a tumor cell ligand for the activating natural killer cell receptor NKp30 in humans," *Journal of Experimental Medicine*, vol. 206, no. 7, pp. 1495–1503, 2009.

[19] O. Mandelboim, N. Lieberman, M. Lev et al., "Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells," *Nature*, vol. 409, no. 6823, pp. 1055–1060, 2001.

[20] O. Hershkovitz, B. Rosental, L. A. Rosenberg et al., "NKp44 receptor mediates interaction of the envelope glycoproteins from the West Nile and dengue viruses with NK cells," *Journal of Immunology*, vol. 183, no. 4, pp. 2610–2621, 2009.

[21] J. Wu, Y. Song, A. B. H. Bakker et al., "An activating immunoreceptor complex formed by NKG2D and DAP10," *Science*, vol. 285, no. 5428, pp. 730–732, 1999.

[22] A. Cerwenka and L. L. Lanier, "Ligands for natural killer cell receptors: redundancy or specificity," *Immunological Reviews*, vol. 181, pp. 158–169, 2001.

[23] A. Diefenbach, E. R. Jensen, A. M. Jamieson, and D. H. Raulet, "Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity," *Nature*, vol. 413, no. 6852, pp. 165–171, 2001.

[24] V. Groh, R. Rhinehart, J. Randolph-Habecker, M. Topp, S. Riddell, and T. Spies, "Costimulation of CD8alphabeta T cell by NKG2D via engagement by MIC induced on virus-infected cells," *Nature Immunology*, vol. 2, no. 3, pp. 255–260, 2001.

[25] C. L. Sutherland, N. J. Chalupny, and D. Cosman, "The UL16-binding proteins, a novel family of MHC class I-related ligands for NKG2D, activate natural killer cell functions," *Immunological Reviews*, vol. 181, pp. 185–192, 2001.

[26] J. Ward, M. Bonaparte, J. Sacks et al., "HIV modulates the expression of ligands important in triggering natural killer cell cytotoxic responses on infected primary T-cell blasts," *Blood*, vol. 110, no. 4, pp. 1207–1214, 2007.

[27] S. Parolini, C. Bottino, M. Falco et al., "X-linked lymphoproliferative disease," *Journal of Experimental Medicine*, vol. 192, no. 3, pp. 337–346, 2000.

[28] C. Bottino, M. Falco, S. Parolini et al., "NTB-A [correction of GNTB-A], a novel SH2D1A-associated surface molecule contributing to the inability of natural killer cells to kill Epstein-Barr virus-infected B cells in X-linked lymphoproliferative disease," *Journal of Experimental Medicine*, vol. 194, no. 3, pp. 235–246, 2001.

[29] M. Vitale, M. Falco, R. Castriconi et al., "Identification of NKp80, a novel triggering molecule expressed by human NK cells," *European Journal of Immunology*, vol. 31, no. 1, pp. 233–242, 2001.

[30] S. Kuttruff, S. Koch, A. Kelp, G. Pawelec, H. G. Rammensee, and A. Steinle, "NKp80 defines and stimulates a reactive subset of CD8 T cells," *Blood*, vol. 113, no. 2, pp. 358–369, 2009.

[31] S. Welte, S. Kuttruff, I. Waldhauer, and A. Steinle, "Mutual activation of natural killer cells and monocytes mediated by NKp80-AICL interaction," *Nature Immunology*, vol. 7, no. 12, pp. 1334–1342, 2006.

[32] C. Bottino, R. Castriconi, D. Pende et al., "Identification of PVR (CD155) and Nectin-2 (CD112) as cell surface ligands for the human DNAM-1 (CD226) activating molecule," *Journal of Experimental Medicine*, vol. 198, no. 4, pp. 557–567, 2003.

[33] J. S. Orange, B. Wang, C. Terhorst, and C. A. Biron, "Requirement for natural killer cell-produced interferon γ in defense against murine cytomegalovirus infection and enhancement of this defense pathway by interleukin 12 administration,"

Journal of Experimental Medicine, vol. 182, no. 4, pp. 1045–1056, 1995.

[34] C. H. Tay, E. Szomolanyi-Tsuda, and R. M. Welsh, "Control of infections by NK cells," *Current Topics in Microbiology and Immunology*, vol. 230, pp. 193–220, 1998.

[35] D. L. Doolan and S. L. Hoffman, "IL-12 and NK cells are required for antigen-specific adaptive immunity against malaria initiated by CD8+ T cells in the plasmodium yoelii model," *Journal of Immunology*, vol. 163, no. 2, pp. 884–892, 1999.

[36] L. L. Ma, G. G. Neely, S. Epelman, A. M. Krensky, and C. H. Mody, "NK cells use perforin rather than granulysin for anticryptococcal activity," *Journal of Immunology*, vol. 173, no. 5, pp. 3357–3365, 2004.

[37] H. Arase, E. S. Mocarski, A. E. Campbell, A. B. Hill, and L. L. Lanier, "Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors," *Science*, vol. 296, no. 5571, pp. 1323–1326, 2002.

[38] L. L. Lanier, "On guard—Activating NK cell receptors," *Nature Immunology*, vol. 2, no. 1, pp. 23–27, 2001.

[39] S. I. Khakoo, C. L. Thio, M. P. Martin et al., "HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection," *Science*, vol. 305, no. 5685, pp. 872–874, 2004.

[40] T. I. Arnon, M. Lev, G. Katz, Y. Chernobrov, A. Porgador, and O. Mandelboim, "Recognition of viral hemagglutinins by NKp44 but not by NKp30," *European Journal of Immunology*, vol. 31, no. 9, pp. 2680–2689, 2001.

[41] R. Gazit, R. Gruda, M. Elboim et al., "Lethal influenza infection in the absence of the natural killer cell receptor gene Ncr1," *Nature Immunology*, vol. 7, no. 5, pp. 517–523, 2006.

[42] S. E. Chisholm and H. T. Reyburn, "Recognition of vaccinia virus-infected cells by human natural killer cells depends on natural cytotoxicity receptors," *Journal of Virology*, vol. 80, no. 5, pp. 2225–2233, 2006.

[43] S. Esin, G. Batoni, C. Counoupas et al., "Direct binding of human NK cell natural cytotoxicity receptor NKp44 to the surfaces of mycobacteria and other bacteria," *Infection and Immunity*, vol. 76, no. 4, pp. 1719–1727, 2008.

[44] V. Vieillard, J. L. Strominger, and P. Debré, "NK cytotoxicity against CD4+ T cells during HIV-1 infection: a gp41 peptide induces the expression of an NKp44 ligand," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 31, pp. 10981–10986, 2005.

[45] V. Vieillard, D. Costagliola, A. Simon, and P. Debré, "Study FAàLTA. Specific adaptive humoral response against a gp41 motif inhibits CD4 T-cell sensitivity to NK lysis during HIV-1 infection," *AIDS*, vol. 20, no. 14, pp. 1795–1804, 2006.

[46] H. Fausther-Bovendo, V. Vieillard, S. Sagan, G. Bismuth, and P. Debré, "Hiv gp41 engages gc1qr on cd4+ t cells to induce the expression of an nk ligand through the pip3/h2o2 pathway," *PLoS Pathogens*, vol. 6, no. 7, Article ID e1000975, pp. 1–14, 2010.

[47] S. Bauer, V. Groh, J. Wu et al., "Activation of NK cells and T cells by NKG2D, a receptor for stress- inducible MICA," *Science*, vol. 285, no. 5428, pp. 727–729, 1999.

[48] M. Kubin, L. Cassiano, J. Chalupny et al., "ULBP1, 2, 3: novel MHC class I-related molecules that bind to human cytomegalovirus glycoprotein UL16, activate NK cells," *European Journal of Immunology*, vol. 31, no. 5, pp. 1428–1437, 2001.

[49] D. Cosman, J. Müllberg, C. L. Sutherland et al., "ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor," *Immunity*, vol. 14, no. 2, pp. 123–133, 2001.

[50] V. Groh, S. Bahram, S. Bauer, A. Herman, M. Beauchamp, and T. Spies, "Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 22, pp. 12445–12450, 1996.

[51] V. Groh, R. Rhinehart, H. Secrist, S. Bauer, K. H. Grabstein, and T. Spies, "Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 12, pp. 6879–6884, 1999.

[52] M. Fang, L. L. Lanier, and L. J. Sigal, "A role for NKG2D in NK cell-mediated resistance to poxvirus disease," *PLoS Pathogens*, vol. 4, no. 2, Article ID e30, 2008.

[53] J. A. Campbell, D. S. Trossman, W. M. Yokoyama, and L. N. Carayannopoulos, "Zoonotic orthopoxviruses encode a high-affinity antagonist of NKG2D," *Journal of Experimental Medicine*, vol. 204, no. 6, pp. 1311–1317, 2007.

[54] P. Costa, S. Sivori, F. Bozzano et al., "IFN-α-mediated increase in cytolytic activity of maturing NK cell upon exposure to HSV-infected myelomonocytes," *European Journal of Immunology*, vol. 39, no. 1, pp. 147–158, 2009.

[55] C. Cerboni, F. Neri, N. Casartelli et al., "Human immunodeficiency virus 1 Nef protein downmodulates the ligands of the activating receptor NKG2D and inhibits natural killer cell-mediated cytotoxicity," *Journal of General Virology*, vol. 88, no. 1, pp. 242–250, 2007.

[56] M. Thomas, J. M. Boname, S. Field et al., "Down-regulation of NKG2D and NKp80 ligands by Kaposi's sarcoma-associated herpesvirus K5 protects against NK cell cytotoxicity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 5, pp. 1656–1661, 2008.

[57] C. Dunn, N. J. Chalupny, C. L. Sutherland et al., "Human cytomegalovirus glycoprotein UL16 causes intracellular sequestration of NKG2D ligands, protecting against natural killer cell cytotoxicity," *Journal of Experimental Medicine*, vol. 197, no. 11, pp. 1427–1439, 2003.

[58] C. Wen, X. He, H. Ma et al., "Hepatitis C virus infection downregulates the ligands of the activating receptor NKG2D," *Cellular & Molecular Immunology*, vol. 5, no. 6, pp. 475–478, 2008.

[59] M. Z. Kubin, D. L. Parshley, W. Din et al., "Molecular cloning and biological characterization of NK cell activation-inducing ligand, a counterstructure for CD48," *European Journal of Immunology*, vol. 29, no. 11, pp. 3466–3477, 1999.

[60] H. Nakajima, M. Cella, H. Langen, A. Friedlein, and M. Colonna, "Activating interactions in human NK cell recognition: the role of 2B4-CD48," *European Journal of Immunology*, vol. 29, no. 5, pp. 1676–1683, 1999.

[61] M. Carrington and S. J. O'Brien, "The Influence of HLA genotype on AIDS," *Annual Review of Medicine*, vol. 54, pp. 535–551, 2003.

[62] M. L. Newell, D. Dunn, A. De Maria et al., "Detection of virus in vertically exposed HIV-antibody-negative children," *The Lancet*, vol. 347, no. 8996, pp. 213–215, 1996.

[63] S. L. Rowland-Jones, D. F. Nixon, M. C. Aldhous et al., "HIV-specific cytotoxic T-cell activity in an HIV-exposed but uninfected infants," *The Lancet*, vol. 341, no. 8849, pp. 860–861, 1993.

[64] A. De Maria, C. Cirillo, and L. Moretta, "Occurrence of human immunodeficiency virus type 1 (HIV-1)-specific cytolytic T cell activity in apparently uninfected children born to

HIV-1- infected mothers," *Journal of Infectious Diseases*, vol. 170, no. 5, pp. 1296–1299, 1994.

[65] R. Kaul, F. Plummer, M. Clerici, M. Bomsel, L. Lopalco, and K. Broliden, "Mucosal IgA in exposed, uninfected subjects: evidence for a role in protection against HIV infection," *AIDS*, vol. 15, no. 3, pp. 431–432, 2001.

[66] G. Poli, M. Introna, and F. Zanaboni, "Natural killer cells in intravenous drug abusers with lymphadenopathy syndrome," *Clinical and Experimental Immunology*, vol. 62, no. 1, pp. 128–135, 1985.

[67] A. De Maria, M. Fogli, P. Costa et al., "The impaired NK cell cytolytic function in viremic HIV-1 infection is associated with a reduced surface expression of natural cytotoxicity receptors (NKp46, NKp30 and NKp44)," *European Journal of Immunology*, vol. 33, no. 9, pp. 2410–2418, 2003.

[68] G. Alter, N. Teigen, B. T. Davis et al., "Sequential deregulation of NK cell subset distribution and function starting in acute HIV-1 infection," *Blood*, vol. 106, no. 10, pp. 3366–3369, 2005.

[69] B. G. Brenner, A. Dascal, R. G. Margolese, and M. A. Wainberg, "Natural killer cell function in patients with acquired immunodeficiency syndrome and related diseases," *Journal of Leukocyte Biology*, vol. 46, no. 1, pp. 75–83, 1989.

[70] M. B. Lucia, N. Froio, E. Tacconelli et al., "CD16+CD56+ CD8+ natural killer (NK) cells are decreased during HIV infection," *European Journal of Histochemistry*, vol. 41, supplement 2, pp. 197–198, 1997.

[71] M. R. Goodier, N. Imami, G. Moyle, B. Gazzard, and F. Gotch, "Loss of the CD56hiCD16- NK cell subset and NK cell interferon-gamma production during antiretroviral therapy for HIV-1: partial recovery by human growth hormone," *Clinical and Experimental Immunology*, vol. 134, no. 3, pp. 470–476, 2003.

[72] D. Mavilio, G. Lombardo, J. Benjamin et al., "Characterization of CD56–/CD16+ natural killer (NK) cells: A highly dysfunctional NK subset expanded in HIV-infected viremic individuals," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 8, pp. 2886–2891, 2005.

[73] M. Fogli, P. Costa, G. Murdaca et al., "Significant NK cell activation associated with decreased cytolytic function in peripheral blood of HIV-1-infected patients," *European Journal of Immunology*, vol. 34, no. 8, pp. 2313–2321, 2004.

[74] D. Mavilio, G. Lombardo, A. Kinter et al., "Characterization of the defective interaction between a subset of natural killer cells and dendritic cells in HIV-1 infection," *Journal of Experimental Medicine*, vol. 203, no. 10, pp. 2339–2350, 2006.

[75] G. Ferlazzo, B. Morandi, A. D'Agostino et al., "The interaction between NK cells and dendritic cells in bacterial infections results in rapid induction of NK cell activation and in the lysis of uninfected dendritic cells," *European Journal of Immunology*, vol. 33, no. 2, pp. 306–313, 2003.

[76] G. Ferlazzo, M. Tsang, L. Moretta, G. Melioli, R. Steinman, and C. Münz, "Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells," *Journal of Experimental Medicine*, vol. 195, no. 3, pp. 343–351, 2002.

[77] D. Pende, R. Castriconi, P. Romagnani et al., "Expression of the DNAM-1 ligands, Nectin-2 (CD112) and poliovirus receptor (CD155), on dendritic cells: relevance for natural killer-dendritic cell interaction," *Blood*, vol. 107, no. 5, pp. 2030–2036, 2006.

[78] G. M. Spaggiari, R. Carosio, D. Pende et al., "NK cell-mediated lysis of autologous antigen-presenting cells is triggered by the engagement of the phosphatidylinositol 3-kinase upon ligation of the natural cytotoxicity receptors NKp30 and NKp46," *European Journal of Immunology*, vol. 31, no. 6, pp. 1656–1665, 2001.

[79] M. Vitale, M. Della Chiesa, S. Carlomagno et al., "NK-dependent DC maturation is mediated by TNF-a and IFN-g released upon engagement of the NKp30 triggering receptor," *Blood*, vol. 106, no. 2, pp. 566–571, 2005.

[80] R. M. Steinman, D. Hawiger, and M. C. Nussenzweig, "Tolerogenic dendritic cells," *Annual Review of Immunology*, vol. 21, pp. 685–711, 2003.

[81] P. Mantegani, G. Tambussi, L. Galli, C. T. Din, A. Lazzarin, and C. Fortis, "Perturbation of the natural killer cell compartment during primary human immunodeficiency virus 1 infection primarily involving the CD56bright subset," *Immunology*, vol. 129, no. 2, pp. 220–233, 2010.

[82] A. De Maria, D. Mavilio, P. Costa, P. Dignetti, M. Fogli, and M. C. Mingari, "Multiple HLA-class I-specific inhibitory NK receptor expression and IL-4/IL-5 production by CD8+ T-cell clones in HIV-1 infection," *Immunology Letters*, vol. 72, no. 3, pp. 179–182, 2000.

[83] D. Mavilio, J. Benjamin, M. Daucher et al., "Natural killer cells in HIV-1 infection: dichotomous effects of viremia on inhibitory and activating receptors and their functional correlates," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 25, pp. 15011–15016, 2003.

[84] A. De Maria, A. Ferraris, M. Guastella et al., "Expression of HLA class I-specific inhibitory natural killer cell receptors in HIV-specific cytolytic T lymphocytes: Impairment of specific cytolytic functions," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 19, pp. 10285–10288, 1997.

[85] A. Valentin, M. Rosati, D. J. Patenaude et al., "Persistent HIV-1 infection of natural killer cells in patients receiving highly active antiretroviral therapy," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 10, pp. 7015–7020, 2002.

[86] H. B. Bernstein, G. Wang, M. C. Plasterer et al., "CD4+ NK cells can be productively infected with HIV, leading to downregulation of CD4 expression and changes in function," *Virology*, vol. 387, no. 1, pp. 59–66, 2009.

[87] J. M. Milush, B. R. Long, J. E. Snyder-Cappione et al., "Functionally distinct subsets of human NK cells and monocyte/DC-like cells identified by coexpression of CD56, CD7, and CD4," *Blood*, vol. 114, no. 23, pp. 4823–4831, 2009.

[88] L. Azzoni, E. Papasavvas, J. Chehimi et al., "Sustained impairment of IFN-gamma secretion in suppressed HIV-infected patients despite mature NK cell recovery: evidence for a defective reconstitution of innate immunity," *Journal of Immunology*, vol. 168, no. 11, pp. 5764–5770, 2002.

[89] F. Bozzano, M. Nasi, L. Bertoncelli et al., "NK-cell phenotype at interruption underlies widely divergent duration of CD4+-guided antiretroviral treatment interruption," *International Immunology*, vol. 23, no. 2, pp. 109–118, 2011.

[90] G. Alter, M. P. Martin, N. Teigen et al., "Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes," *Journal of Experimental Medicine*, vol. 204, no. 12, pp. 3027–3036, 2007.

[91] M. P. Martin, X. Gao, J. H. Lee et al., "Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS," *Nature Genetics*, vol. 31, no. 4, pp. 429–434, 2002.

[92] A. De Maria and L. Moretta, "NK cell function in HIV-1 infection," *Current HIV Research*, vol. 6, no. 5, pp. 433–440, 2008.

[93] A. De Maria, R. Biassoni, M. Fogli et al., "Identification, molecular cloning and functional characterization of NKp46 and NKp30 natural cytotoxicity receptors in Macaca fascicularis NK cells," *European Journal of Immunology*, vol. 31, no. 12, pp. 3546–3556, 2001.

[94] R. Biassoni, M. Fogli, C. Cantoni et al., "Molecular and functional characterization of NKG2D, NKp80, and NKG2C triggering NK cell receptors in rhesus and cynomolgus macaques: monitoring of NK cell function during simian HIV infection," *Journal of Immunology*, vol. 174, no. 9, pp. 5695–5705, 2005.

[95] A. De Maria, E. Ugolotti, E. Rutjens et al., "NKp44 expression, phylogenesis and function in non-human primate NK cells," *International Immunology*, vol. 21, no. 3, pp. 245–255, 2009.

[96] E. Rutjens, S. Mazza, R. Biassoni et al., "Differential NKp30 inducibility in chimpanzee NK cells and conserved NK cell phenotype and function in long-term HIV-1-infected animals," *Journal of Immunology*, vol. 178, no. 3, pp. 1702–1712, 2007.

[97] J. Corado, F. Toro, H. Rivera, N. E. Bianco, L. Deibis, and J. B. De Sanctis, "Impairment of natural killer (NK) cytotoxic activity in hepatitis C virus (HCV) infection," *Clinical and Experimental Immunology*, vol. 109, no. 3, pp. 451–457, 1997.

[98] M. G. Lassen, J. R. Lukens, J. S. Dolina, M. G. Brown, and Y. S. Hahn, "Intrahepatic IL-10 maintains NKG2A+Ly49- liver NK cells in a functionally hyporesponsive state," *Journal of Immunology*, vol. 184, no. 5, pp. 2693–2701, 2010.

[99] B. Amadei, S. Urbani, A. Cazaly et al., "Activation of natural killer cells during acute infection with hepatitis C virus," *Gastroenterology*, vol. 138, no. 4, pp. 1536–1545, 2010.

[100] S. Pelletier, C. Drouin, N. Bédard, S. I. Khakoo, J. Bruneau, and N. H. Shoukry, "Increased degranulation of natural killer cells during acute HCV correlates with the magnitude of virus-specific T cell responses," *Journal of Hepatology*, vol. 53, no. 5, pp. 805–816, 2010.

[101] C. Morishima, D. M. Paschal, C. C. Wang et al., "Decreased NK cell frequency in chronic hepatitis C does not affect ex vivo cytolytic killing," *Hepatology*, vol. 43, no. 3, pp. 573–580, 2006.

[102] P. Bonorino, M. Ramzan, X. Camous et al., "Fine characterization of intrahepatic NK cells expressing natural killer receptors in chronic hepatitis B and C," *Journal of Hepatology*, vol. 51, no. 3, pp. 458–467, 2009.

[103] U. C. Meier, R. E. Owen, E. Taylor et al., "Shared alterations in NK cell frequency, phenotype, and function in chronic human immunodeficiency virus and hepatitis C virus infections," *Journal of Virology*, vol. 79, no. 19, pp. 12365–12374, 2005.

[104] B. Oliviero, S. Varchetta, E. Paudice et al., "Natural killer cell functional dichotomy in chronic hepatitis B and chronic hepatitis C virus infections," *Gastroenterology*, vol. 137, no. 3, pp. 1151–1160, 2009.

[105] O. Dessouki, Y. Kamiya, H. Nagahama et al., "Chronic hepatitis C viral infection reduces NK cell frequency and suppresses cytokine secretion: reversion by anti-viral treatment," *Biochemical and Biophysical Research Communications*, vol. 393, no. 2, pp. 331–337, 2010.

[106] L. Golden-Mason, L. Madrigal-Estebas, E. McGrath et al., "Altered natural killer cell subset distributions in resolved and persistent hepatitis C virus infection following single source exposure," *Gut*, vol. 57, no. 8, pp. 1121–1128, 2008.

[107] M. S. Bonavita, A. Franco, M. Paroli et al., "Normalization of depressed natural killer activity after interferon-α therapy is associated with a low frequency of relapse in patients with chronic hepatitis C," *International Journal of Tissue Reactions*, vol. 15, no. 1, pp. 11–16, 1993.

[108] A. De Maria, M. Fogli, S. Mazza et al., "Increased natural cytotoxicity receptor expression and relevant IL-10 production in NK cells from chronically infected viremic HCV patients," *European Journal of Immunology*, vol. 37, no. 2, pp. 445–455, 2007.

[109] M. Jinushi, T. Takehara, T. Tatsumi et al., "Negative regulation of NK cell activities by inhibitory receptor CD94/NKG2A leads to altered NK cell-induced modulation of dendritic cell functions in chronic hepatitis C virus infection," *Journal of Immunology*, vol. 173, no. 10, pp. 6072–6081, 2004.

[110] T. Takehara and N. Hayashi, "Natural killer cells in hepatitis C virus infection: from innate immunity to adaptive immunity," *Clinical Gastroenterology and Hepatology*, vol. 3, no. 10 ,supplement 2, pp. S78–S81, 2005.

[111] G. Ahlenstiel, R. H. Titerence, C. Koh et al., "Natural killer cells are polarized toward cytotoxicity in chronic hepatitis C in an interferon-alfa-dependent manner," *Gastroenterology*, vol. 138, no. 1, pp. 325–335, 2010.

[112] R. J. Harrison, A. Ettorre, A. M. Little, and S. I. Khakoo, "Association of NKG2A with treatment for chronic hepatitis C virus infection," *Clinical and Experimental Immunology*, vol. 161, no. 2, pp. 306–314, 2010.

[113] J. Nattermann, G. Feldmann, G. Ahlenstiel, B. Langhans, T. Sauerbruch, and U. Spengler, "Surface expression and cytolytic function of natural killer cell receptors is altered in chronic hepatitis C," *Gut*, vol. 55, no. 6, pp. 869–877, 2006.

[114] F. Bozzano, A. Picciotto, P. Costa et al., "Activating NK cell receptor expression/function (NKp30,NKp46,DNAM-1) during chronic viraemic HCV infection is associated with the outcome of combined treatment," *European Journal of Immunology*, 2011.

[115] J. C. Yoon, M. Shiina, G. Ahlenstiel, and B. Rehermann, "Natural killer cell function is intact after direct exposure to infectious hepatitis C virions," *Hepatology*, vol. 49, no. 1, pp. 12–21, 2009.

[116] S. Crotta, M. Brazzoli, D. Piccioli, N. M. Valiante, and A. Wack, "Hepatitis C virions subvert natural killer cell activation to generate a cytokine environment permissive for infection," *Journal of Hepatology*, vol. 52, no. 2, pp. 183–190, 2010.

[117] S. Knapp, U. Warshow, D. Hegazy et al., "Consistent beneficial effects of killer cell immunoglobulin-like receptor 2dl3 and group 1 human leukocyte antigen-c following exposure to hepatitis c virus," *Hepatology*, vol. 51, no. 4, pp. 1168–1175, 2010.

[118] E. Marcenaro, B. Ferranti, M. Falco, L. Moretta, and A. Moretta, "Human NK cells directly recognize Mycobacterium bovis via TLR2 and acquire the ability to kill monocyte-derived DC," *International Immunology*, vol. 20, no. 9, pp. 1155–1167, 2008.

[119] R. Vankayalapati, B. Wizel, S. E. Weis et al., "The NKp46 receptor contributes to NK cell lysis of mononuclear phagocytes infected with an intracellular bacterium," *Journal of Immunology*, vol. 168, no. 7, pp. 3451–3457, 2002.

[120] L. E. Bermudez, M. Wu, and L. S. Young, "Interleukin-12-stimulated natural killer cells can activate human macrophages to inhibit growth of Mycobacterium avium," *Infection and Immunity*, vol. 63, no. 10, pp. 4099–4104, 1995.

[121] K. J. Brill, Q. Li, R. Larkin et al., "Human natural killer cells mediate killing of intracellular Mycobacterium tuberculosis H37Rv via granule-independent mechanisms," *Infection and Immunity*, vol. 69, no. 3, pp. 1755–1765, 2001.

[122] P. Katz, H. Yeager Jr., G. Whalen, M. Evans, R. P. Swartz, and J. Roecklein, "Natural killer cell-mediated lysis of Mycobacterium-avium complex-infected monocytes," *Journal of Clinical Immunology*, vol. 10, no. 1, pp. 71–77, 1990.

[123] M. Lichtner, R. Rossi, F. Mengoni et al., "Circulating dendritic cells and interferon-α production in patients with tuberculosis: Correlation with clinical outcome and treatment response," *Clinical and Experimental Immunology*, vol. 143, no. 2, pp. 329–337, 2006.

[124] J. Hinchey, S. Lee, B. Y. Jeon et al., "Enhanced priming of adaptive immunity by a proapoptotic mutant of Mycobacterium tuberculosis," *Journal of Clinical Investigation*, vol. 117, no. 8, pp. 2279–2288, 2007.

[125] R. Vankayalapati, P. Klucar, B. Wizel et al., "NK cells regulate CD8+ T cell effector function in response to an intracellular pathogen," *Journal of Immunology*, vol. 172, no. 1, pp. 130–137, 2004.

[126] F. Bozzano, P. Costa, G. Passalacqua et al., "Functionally relevant decreases in activatory receptor expression on NK cells are associated with pulmonary tuberculosis in vivo and persist after successful treatment," *International Immunology*, vol. 21, no. 7, pp. 779–791, 2009.

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Review Article

The Role of Natural Killer Cells in Sepsis

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Severe sepsis and septic shock are still deadly conditions urging to develop novel therapies. A better understanding of the complex modifications of the immune system of septic patients is needed for the development of innovative immunointerventions. Natural killer (NK) cells are characterized as $CD3^{-}NKp46^{+}CD56^{+}$ cells that can be cytotoxic and/or produce high amounts of cytokines such as IFN-γ. NK cells are also engaged in crosstalks with other immune cells, such as dendritic cells, macrophages, and neutrophils. During the early stage of septic shock, NK cells may play a key role in the promotion of the systemic inflammation, as suggested in mice models. Alternatively, at a later stage, NK cells-acquired dysfunction could favor nosocomial infections and mortality. Standardized biological tools defining patients' NK cell status during the different stages of sepsis are mandatory to guide potential immuno-interventions. Herein, we review the potential role of NK cells during severe sepsis and septic shock.

1. Introduction

Sepsis is the clinical presentation of a "systemic inflammatory response syndrome" (SIRS) to a severe infection. Most clinical and basic-science research on the immune consequences of severe sepsis conducted during the last decades has focused on the roles of macrophages, neutrophils and conventional T lymphocytes [1]. During recent years, however, it has become increasingly clear that subsets of innate immune cells, such as natural killer (NK) cells, are involved in both protective immunity and immunopathology.

Herein, we review the potential role of NK cells during the different stages of severe sepsis and septic shock.

2. Severe Sepsis: Urgent Needs for "Immunological" Solutions

The most threatening infections are referred to as severe sepsis and septic shock [1]. These severe forms of infection, mainly of bacterial origin, represent a major health-care problem, accounting for thousands of deaths every year worldwide, with more than 200,000 deaths per year just in the United States [2]. Sepsis, severe sepsis, and septic shock are viewed as a continuum that results in increasing mortality (cf. Figure 1) and shares consensual clinical criteria [3]. Mortality is up to 50% in septic shock, and the incidence of sepsis is projected to increase

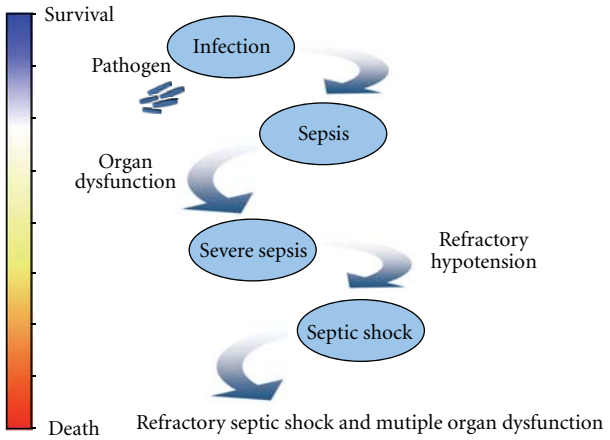

Figure 1: Continuum from infection to septic shock: the initial response to pathogen is a systemic response, with release of inflammatory mediators and activation of the coagulation cascade, resulting in imbalance between oxygen delivery and oxygen consumption. Ultimately, tissue hypoxia develops and may lead to multiple organ dysfunction and irreversible shock.

significantly during next years with higher rates of mortality due to more advanced age and/or associated comorbidities (cancer, diabetes, etc.). During the last decades, physicians have made significant progress in the early implementation of symptomatic care through adequate fluid resuscitation, antibiotherapy, and specific organ-support techniques, such as mechanical ventilation and renal-replacement therapy. Unfortunately, these therapeutic strategies have failed to sufficiently reduce mortality in severely septic patients [4, 5]. Moreover, physicians are increasingly concerned about increasing microbial resistance to antibiotics and the slow development of new antimicrobial agents [6]. Thus, there is an urgent need to develop efficacious therapies to treat this deadly disease.

Future therapies may emerge from a better understanding of the physiopathology of sepsis [7]. Sepsis, also referred to as SIRS of septic origin, was originally viewed as an exacerbated inflammatory response and a "cytokine storm." However, most trials that used inhibitors of proinflammatory cytokines or inhibitors of proinflammatory mediators failed to improve patients' outcomes, providing the best proof of the incomplete understanding of its pathogenesis [8, 9]. One of the reasons for the lack of efficacy of anti-inflammatory strategies in patients with sepsis may be because the syndrome changes over time [10].

In its early stages, sepsis is characterized by an increase in inflammatory mediators, but as sepsis persists, there is a shift towards an anti-inflammatory immunosuppressive state. Indeed, a common feature of these patients is the alteration of their immune status, referred to as "compensatory anti-inflammatory response syndrome" (CARS), which is thought to render patients more susceptible to nosocomial infections. It seems that immune dysfunctions that are supposed to play a role in mortality vary between patients who succumb within the first hour after sepsis and those who survive the first critical hours (>80%) but then die later from sepsis-induced multiorgan dysfunction and/or secondary nosocomial infections. It has only been recently that efforts to understand the effect of the inflammatory process on the immune status during septic shock have fully integrated the considerable derangements of both the innate and adaptive immune systems and have better identified the contribution of multiple cellular actors [1, 7].

3. NK Cells: Early Soldiers with Multiple Functions

NK cells are lymphocytes that are classically referred to as part of the innate immunity. NK cells were first described for their ability to kill leukemic cells without prior specific sensitization [11]. They represent a small proportion (4–15%) of blood lymphocytes and do not express a specific receptor for antigens dependent upon RAG-mediated rearrangements [12]. NK cell function is regulated by a multiplicity of activating and inhibitory receptors. Their natural cytotoxicity is largely under the control of natural cytotoxicity receptors, and their antibody-dependent cytotoxicity is linked to the engagement of CD16/$F_C\gamma$ RIIIa [13]. Human NK cells are characterized as $CD3^-$ $NKp46^+CD56^+$ cells [14]. In humans, blood NK cells can be divided into two major subtypes: $CD56^{bright}$ and $CD56^{dim}$, corresponding to sequential steps of differentiation [15]. The former subtype represents about 10% of circulating NK cells. These cells express low levels of CD16 and perforin, produce high amounts of cytokines (e.g., interferon gamma or IFN-γ, TNF-α, and granulocyte-macrophage colony-stimulating factor) in response to cytokines such as interleukin (IL)-12 and IL-18, and represent the major fraction of NK cells in lymph nodes. $CD56^{dim}$ NK cells express high levels of CD16, perforin, and killer Ig-like receptors (KIRs). KIRs include inhibitory receptors that recognize MHC class I molecules and dampen NK cell activation. $CD56^{dim}$ NK cells are cytotoxic by granule polarization and exocytosis of various proteins including perforin and granzymes, which mediate target-cell killing and are also cytokine producers.

Several lines of evidence suggest that NK cells might be involved in key functions during sepsis. NK cells have a major role in defense against viral infections, in particular herpesvirus [16], influenza viruses [17], or hantavirus [18], by direct cytotoxicity against virus-infected cells and by the early production of cytokines that can control viral replication, such as IFN-γ. NK cells also participate in responses to other types of infections, including those caused by intracellular bacteria, pyogenic bacteria, fungi, and protozoa [19, 20]. As the early and main producers of IFN-γ during sepsis, these cells are equipped with many innate sensors for damage-associated molecular-pattern molecules (DAMPS) and pathogen-associated molecular-pattern molecules (PAMPS) [21]. In addition, if NK cells are found within the blood stream, they are also abundant in some tissues, such as the lungs [22, 23], an organ particularly prone to dysfunction in Intensive Care Unit (ICU) patients. NK cells are also engaged in crosstalks with other immune cells, such as dendritic cells (DCs) [24], monocytes,

macrophages [25, 26], and neutrophils [27], which besides being fundamental for NK cell activation in response to most pathogens (by direct contact or cytokine secretion) also participate in the development of the subsequent immune response (Figures 2(A) and 2(B)).

4. NK Cells and Severe Sepsis: Lessons and Limits from Murine Models

Most of the current knowledge about the role of NK cells during severe sepsis comes from mouse models. Although NK cell-deficient mice are not reported to present with detectable abnormalities at steady state, all data converge on a detrimental role for NK cells during sepsis. In mice, a challenge with high doses of lipopolysaccharide (LPS) results in a syndrome resembling septic shock in humans, and depletion of NK cells offers protection against LPS-induced shock [28, 29]. Depletion of NK cells by systemic administration of polyclonal antiasialo GM1 or monoclonal anti-NK1.1 antibodies, before the induction of the generalized Schwartzman reaction, leads to a dramatic reduction in mortality and significantly lowers cytokine levels (IFN-γ and TNF-α) following a systemic injection of LPS [28]. The same protective effect against cytokine-induced shock (by administrating IL-12 in combination with IL-2 or IL-15) was observed in mice that underwent depletion of NK cells with antiasialo GM1 antibodies [30].

In addition, there is now increasing evidence of detrimental roles for NK cells in different models of bacterial infections. Depletion of NK cells in SCID mice infected intranasally with *Streptococcus pneumoniae* resulted in significantly lower bacteremia and inflammatory cytokine production within the lung airways and lung tissue [31]. Improved survival was also observed with NK-cell-depleted mice in a model of septic shock with *Streptococcus pneumoniae* [32]. In a model of cecal ligation and puncture (CLP), mice treated with anti-asialo-GM1 were protected against CLP-induced mortality compared to IgG-treated controls [32]. During CLP-induced shock, NK cells migrated from blood and spleen to the inflamed peritoneal cavity where they amplified the proinflammatory activities of the myeloid cell populations [33]. NK cells were also involved in the high levels of inflammatory cytokines, lung pathology, and mortality that occur during *Escherichia coli* peritonitis, as all these parameters were reduced by NK depletion [34].

Altogether, these results suggest that NK cells can promote the inflammatory process occurring during sepsis *in vivo*, possibly via interactions with macrophages [35, 36], organ infiltration and damage, and the secretion of proinflammatory cytokines, providing a rationale basis to explain how NK-cell depletion increases survival in experimental sepsis. In opposition to this role of amplification of inflammation, recent data show that very early during the course of systemic infections induced by *Toxoplasma gondii*, *Listeria monocytogenes*, and *Yersinia pestis*, IL-12 secreted by DC induces NK cells to produce the broadly immunosuppressive cytokine IL-10, which, in turn, inhibits IL-12 secretion by DC, unveiling an immunosuppressive "regulator" function of NK cells [37]. If documented in humans, NK cells might then contribute to the necessary transition from SIRS to CARS (cf. Figure 2).

Mice are the most commonly used animal models in biomedical research, and rodent studies are an important part of the preclinical studies that determine progression to clinical studies in humans during drug development. However, there are numerous concerns about extrapolation from what is known about mouse to human NK-cell biology during severe sepsis, thus limiting the clinical relevance of the mouse models described above. Because there was no genetic model where NK cells could be selectively deleted, *in vivo* NK-cell depletion has so far relied on anti-asialo-GM1- or anti-NK1.1-depleting antibodies. Although a depletion of NK cells can be obtained with both antibodies, the selectivity of the depletion depends upon the quantities of antibodies, blurring the interpretation of the results obtained using these methods. More recently, transgenic mice that lack NK cells, but have a normal T/NKT-cell compartment, have been reported [38], but the cause of selective NK-cell ablation in these mice is linked to the expression of the ubiquitous transcription factor, ATF2, which raises the possibility of other defects in the immune system [39]. Moreover, the basic leucine-zipper transcription factor E4BP4 (also called NFIL3) has been proven essential for the generation of the NK-cell lineage and E4BP4-deficient mice specifically lack NK cells [40]. However, E4BP4-deficient mice were also shown to undergo impaired B-cell intrinsic IgE class switching [41]. Finally, taking advantage of the identification of a functional NKp46 promoter, a mouse model of conditional NK cell ablation based on the diphtheria toxin (DT) receptor/DT-based system has been generated [42]. Because DT injection leads to a complete and selective ablation of NK cells in these mice, this model provides a precious tool to explore the role of NK cells in many pathological conditions, including sepsis.

However, even with the availability of selective NK-cell deficient models [42] or of humanized mouse models [43], a constant problem with the murine approach to study sepsis is that rodents are highly resilient to most models of induced inflammation as compared to humans and that results depend on the strain and models of sepsis used (from LPS injection to cecal ligation and puncture). Also, because the supportive care used in humans is not easily transposed into mice [44], these models of septic challenge are not fully relevant to address the particular situation of ICU patients who survive the most severe sepsis and then come to the "immunosuppressive" CARS stage, which is responsible for most deaths.

5. A Role for NK Cells in Human SIRS?

By analogy with the possible use of the NK cell-deficient mouse model, we could consider the study of patients with NK cell-selective deficiency to address the role of NK cells in severe human sepsis. A number of isolated deficiencies of NK cells in humans have been described, but most are complex immunodeficiencies associated with absent

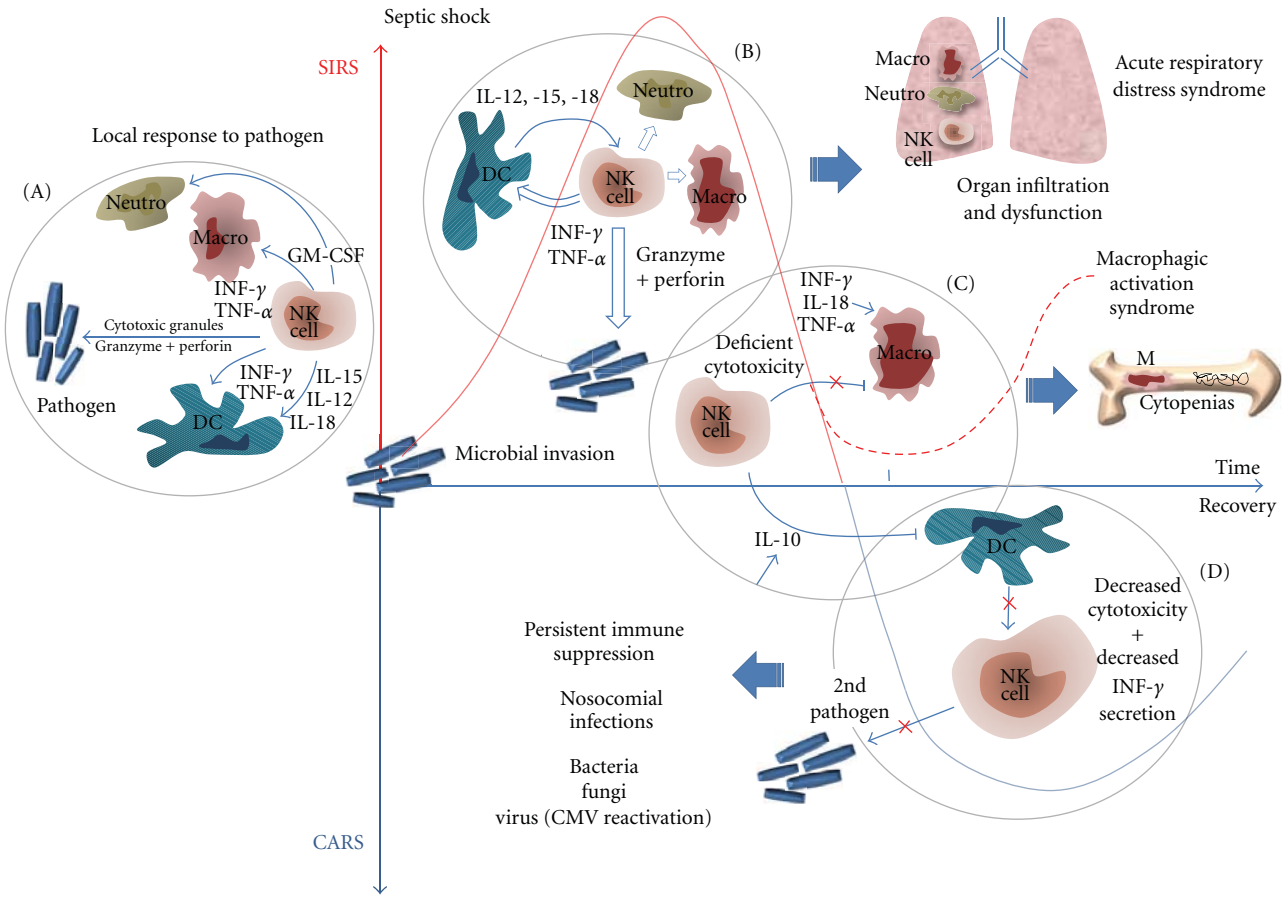

Figure 2: (A) NK cells initiate a local inflammatory response to pathogens. (B) During SIRS, NK cells amplify the inflammatory response to the spread of the pathogen, which can lead to organ dysfunction. (C) Deficient NK cell cytotoxicity may favor macrophage activation syndrome. (D) During CARS, NK cell global dysfunction may favor nosocomial infections. Note: SIRS and CARS have been separated in time to ease understanding of the figure, but the different stages (B–D) can occur simultaneously. Also, most data shown here are from mouse models and should be further confirmed in septic patients.

or functionally deficient NK cells [45]. Few reports have described patients with isolated NK-cell abnormalities where the main susceptibility is to severe infection with herpesvirus [46]. The paucity of nonambiguous cases of NK-selective deficiencies in humans has hampered the identification of nonredundant NK-cell function. Also, as some SCID-X1 patients with no NK cell reconstitution after allogenic bone-marrow transplantation or gene therapy do not experience severe infections, it has been suggested that NK cells might have redundant anti-infectious functions in humans [47]. However, NK cells have been reported to be key in controlling severe cytomegalovirus (CMV) infection in some patients [48]. In septic shock, as fatality can precede adaptive responses, and in the context of massive and sometimes persistent apoptosis-induced T- and B-cell lymphopenia [49], NK cells may play a crucial and nonredundant role.

Our knowledge of NK cells in severe human sepsis and septic shock could be derived from analyses of NK cells taken directly from patients during the different clinical stages of the disease. However, available data from patients in ICU are scarce and heterogeneous and do not always include evaluation of cell function. Because of all these limitations, these results appear as contradictory. Yet, in patients with severe Gram-negative sepsis, an increased percentage of blood NK cells has been reported, as an improved survival in the patients with high NK counts [50]. Of importance, these patients did not experience septic shock nor were admitted into the ICU. Unfortunately, NK cell effector functions were not monitored in this study. A previous report had observed that NK-cell counts were higher among patients with sepsis of Gram-positive origin than among patients with Gram-negative sepsis [51].

In contrast, previous studies on patients with SIRS [52] and septic shock [53] had reported reduced numbers of NK cells and impaired NK cell *in vitro* cytotoxicity against K562 tumor cells. However, when NK cell cytotoxicity in patients with severe sepsis or septic shock was assessed *in vivo* by measuring circulating granzyme A and B levels [54], higher cytotoxicity was found in 50% of septic patients, and these patients had a higher mortality and worse organ function. Altogether, as suggested by a recent prospective study conducted in more than 500 patients with early sepsis,

the discrepancies concerning the number and/or function of circulating NK cells are probably due to the heterogeneity of patients in terms of either severity (severe sepsis and/or septic shock) or involvement of pathogens (Gram-negative versus-positive bacteria) [55].

Also, because septic shock is rapidly associated with a dramatic decrease in circulating lymphocytes, the timing of NK-cell analysis might be of particular importance. It is reported that, from their admission into an ICU, the numbers of all lymphocyte subpopulations (including NK cells) of 21 septic-shock patients were diminished, and these alterations remained stable during the first 48 h [56], while no data are available after this short time.

Another caveat in these human studies is that NK cell testing has been obviously limited to peripheral blood. As NK cells can migrate out of the blood into the inflamed tissues, the interpretation of the analysis may be difficult. Indeed, the status of NK cells within tissues might be quite different [57, 58]. Only one study has addressed NK cells at a tissular level in human septic shock. In this paper, Hotchkiss et al. described a profound and progressive, apoptosis-induced loss of B and $CD4^+$ T cells in the spleen and gut-associated lymphoid tissue of adults who had died of sepsis [49]. In contrast, a trend towards an increase of splenic NK cells was observed in septic patients. This result failed to reach statistical significance, most likely as the consequence of the small number of patients.

Thus, with cautious interpretation, due to the mentioned heterogeneity in studied patients and the complete absence of data concerning NK cell cytokine secretion, human studies do not exclude a detrimental role for NK cells in the early stage of septic shock (Figure 1) that was observed in mouse models [49, 54].

6. A Role for NK Cells in Human CARS?

As the vast majority of patients with sepsis survive the initial insult, we should consider not only the initial excessive systemic inflammatory reaction, but also the following sepsis-induced immunosuppressive period and its consequences.

During severe sepsis, some patients can develop secondary hemophagocytic lymphohistiocytic (HLH) syndrome, also termed macrophage activation syndrome (MAS). For intensivists, the features of MAS mainly include nonremitting fever, severe cytopenias, and organ dysfunctions. MAS is characterized by uncontrolled macrophage and Th1-lymphocyte stimulation, with elevated levels of circulating INF-γ, TNF-α, IL-6, and IL-18 [59]. Many clues to the role of NK cells in MAS have been recently discovered. First, a marked decrease in NK cell numbers, as well as a severe decrease in both natural cytotoxicity and ADCC, has been reported in patients with secondary MAS [60]. Instead of being just a consequence of MAS, this defect of NK cell number and function could be part of the pathogenesis. Indeed, the genetic forms of HLH are characterized by an intrinsic defect of NK cell and T-cell cytotoxicity related to the perforin/granzyme release pathway [61], and virally infected perforin KO mice represent a relevant model of MAS [62]. Interactions between human NK cells and macrophages are bidirectional and can result in activation of NK cells or in the regulation of macrophage activity through the killing of activated macrophages by NK cells [63]. Thus, even if NK cells are early and massive sources of INF-γ, and contribute to the initial excessive inflammatory response in severe sepsis, a concomitant defect of their cytotoxic functions could predispose a subset of these septic patients to develop MAS because NK cell dysfunction may contribute to uncontrolled Th1-lymphocyte and macrophage activation (Figure 2(C)).

Reduced NK cell numbers and functions, if persistent, may also contribute to impaired host defenses during CARS (Figure 2(D)). This "compensatory" inhibitory response, which is primarily seen as a regulation for hyperinflammation, can then become deleterious as many immune functions are compromised. These alterations may be directly responsible for the worsening outcome, as they may play a major role in the decreased resistance to nosocomial infections in patients who have survived an initial resuscitation. In humans, this view is actually merely speculative as up to now only a single study has been reported, which includes an evaluation of NK cells in ICU patients with septic shock that was not restricted to the very early stage of shock [64]. In this study, NK cell cytotoxicity was evaluated at different time points from admission, and it was suppressed to <10% in nearly all patients during the complete observation period (up to 14 days). Interestingly, ICU patients presenting with septic shock seem more prone than others to develop reactivation of CMV [65]. These CMV reactivations occur mainly at the late stage of sepsis although the affected patients seem to have sufficient CMV-specific $CD4^+$ or $CD8^+$ T cells [64, 66]. These data suggest that there might be a decrease in NK cell function that favors the progression of the viral infection. Finally, ICU patients with CMV reactivation (up to 30% of all ICU patients) may develop more bacterial/fungal nosocomial infections because of the immunosuppressive properties of CMV [65], but one cannot exclude the possibility that NK cells also participate in protective immunity against nosocomial pathogens [67].

7. NK Cells and Future Therapies for Severe Human Sepsis: Perspectives

Most of the published data on human sepsis do not examine both NK cell numbers and functions and, thus, have incompletely assessed NK cell status. Nowadays, there are rapid and relatively inexpensive methods to assess NK cell functions directly at the patient bedside, using multiparametric functional flow cytometry [68]. Both quantitative and qualitative evaluation of NK status now needs to be broadly performed among ICU patients.

In addition, a single parameter will likely not be sufficient to characterize the complexity of septic patients' immunological status, which rapidly changes over time. Therefore, the introduction of high-throughput technologies represents an emerging solution for the global immunomonitoring of sepsis. DNA microarrays and RNAseq allow genome-wide assessment of changes in mRNA abundance. A first transcriptomic approach could be restricted to NK-specific

genes. Strikingly, less than a hundred genes might be sufficient to define the human NK cell-specific signature [69]. One should also look for NK cell-specific combinations of more broadly expressed genes during the different phases of severe sepsis. This global functional approach has been recently performed as a modular approach in different human pathological conditions [70, 71].

As for the potential development of any NK-based immunointervention, new approaches should also allow to better define the complex and dynamic actions of NK cells during the different phases of severe sepsis in humans. At present, targeted NK cell therapies address hematopoietic malignancies using different strategies to enhance NK cell functions and promote their antitumor action [72]. These innovative protocols could be used in the "CARS" period when ICU patients suffer from immunosuppression and nosocomial infection. NK cells stimulation could be achieved by manipulation of NK receptors (i.e., using anti-KIR-specific antibodies that block inhibitory receptors) or by the administration of cytokines as IL-15. The last option has proved successful in murine model of sepsis and pneumonia, where administration of IL-15 could prevent apoptosis, increase the percentage of NK cells that produce IFN-γ, and reverse immune dysfunction [73]. The restoration of INF-γ secretion by NK cells that might be able to migrate and deliver cytokine into the infected tissues at the right time might be more efficient than the direct parenteral administration of INF-γ [74]. Alternatively, in the very early phase of severe sepsis, monoclonal antibodies, targeting, for example, NKp46, could be also designed to downregulate or deplete NK cells and prevent the consequences of uncontrolled inflammation due to their gamma interferon secretion. NK cell depletion should be transient to avoid a supplementary "immunodepression" due to persistent NK depletion during CARS. Also, it might be difficult to use it early enough in patients initiating a septic shock out of the hospital; but instead, inpatients, for example, presenting a postsurgical sepsis, could be carefully screened and receive immunointervention at the earliest phase of sepsis or even at a presymptomatic phase of sepsis, if it can be robustly diagnosed [75].

Translation to its clinical application must carefully take into account the timing of administration of immunotherapeutical agent. Thus, one of the first goals should be first to achieve robust and standardized biological tools that accurately define the patient's immune status, so that physicians can decide who can benefit, and when, from those immunointerventions.

Abbreviations

CARS: Compensatory anti-inflammatory response syndrome
CMV: Cytomegalovirus
DCs: Dendritic cells
Macro: Macrophages
NK: Natural killer
Neutro: Neutrophils
SIRS: Systemic inflammatory response syndrome.

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References

[1] R. S. Hotchkiss and I. E. Karl, "The pathophysiology and treatment of sepsis," *New England Journal of Medicine*, vol. 348, no. 2, pp. 138–150, 2003.

[2] D. C. Angus, W. T. Linde-Zwirble, J. Lidicker, G. Clermont, J. Carcillo, and M. R. Pinsky, "Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care," *Critical Care Medicine*, vol. 29, no. 7, pp. 1303–1310, 2001.

[3] R. C. Bone, R. A. Balk, F. B. Cerra et al., "Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine," *Chest*, vol. 101, pp. 1644–1655, 1992.

[4] E. Rivers, B. Nguyen, S. Havstad et al., "Early goal-directed therapy in the treatment of severe sepsis and septic shock," *New England Journal of Medicine*, vol. 345, no. 19, pp. 1368–1377, 2001.

[5] R. P. Dellinger, M. M. Levy, J. M. Carlet et al., "Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2008," *Critical Care Medicine*, vol. 36, no. 1, pp. 296–327, 2008.

[6] S. M. Opal and T. Calandra, "Antibiotic usage and resistance: gaining or losing ground on infections in critically ill patients?" *Journal of the American Medical Association*, vol. 302, no. 21, pp. 2367–2368, 2009.

[7] P. D. Annane, P. E. Bellissant, and J. M. Cavaillon, "Septic shock," *Lancet*, vol. 365, no. 9453, pp. 63–78, 2005.

[8] C. J. Fisher Jr., J. M. Agosti, S. M. Opal et al., "Treatment of septic shock with the tumor necrosis factor receptor: Fc fusion protein," *New England Journal of Medicine*, vol. 334, no. 26, pp. 1697–1702, 1996.

[9] S. M. Opal, C. J. Fisher Jr., J.-F.A. Dhainaut et al., "Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial," *Critical Care Medicine*, vol. 25, no. 7, pp. 1115–1124, 1997.

[10] R. C. Bone, "Immunologic dissonance: a continuing evolution in our understanding of the systemic inflammatory response syndrome (SIRS) and the Multiple Organ Dysfunction Syndrome (MODS)," *Annals of Internal Medicine*, vol. 125, no. 8, pp. 680–687, 1996.

[11] R. B. Herberman, M. E. Nunn, and D. H. Lavrin, "Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. I. Distribution of reactivity and specificity," *International Journal of Cancer*, vol. 16, no. 2, pp. 216–229, 1975.

[12] E. Vivier, E. Tomasello, M. Baratin, T. Walzer, and S. Ugolini, "Functions of natural killer cells," *Nature Immunology*, vol. 9, no. 5, pp. 503–510, 2008.

[13] L. Moretta and A. Moretta, "Unravelling natural killer cell function: triggering and inhibitory human NK receptors," *EMBO Journal*, vol. 23, no. 2, pp. 255–259, 2004.

[14] E. Vivier, D. H. Raulet, A. Moretta et al., "Innate or adaptive immunity? The example of natural killer cells," *Science*, vol. 331, no. 6013, pp. 44–49, 2011.

[15] C. Romagnani, K. Juelke, M. Falco et al., "CD56CD16 killer Ig-like receptor NK cells display longer telomeres and acquire features of CD56 NK cells upon activation," *Journal of Immunology*, vol. 178, no. 8, pp. 4947–4955, 2007.

[16] H. Arase, E. S. Mocarski, A. E. Campbell, A. B. Hill, and L. L. Lanier, "Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors," *Science*, vol. 296, no. 5571, pp. 1323–1326, 2002.

[17] O. Mandelboim, N. Lieberman, M. Lev et al., "Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells," *Nature*, vol. 409, no. 6823, pp. 1055–1060, 2001.

[18] N. K. Björkström, T. Lindgren, M. Stoltz et al., "Rapid expansion and long-term persistence of elevated NK cell numbers in humans infected with hantavirus," *Journal of Experimental Medicine*, vol. 208, no. 1, pp. 13–21, 2011.

[19] C. H. Tay, E. Szomolanyi-Tsuda, and R. M. Welsh, "Control of infections by NK cells," *Current Topics in Microbiology and Immunology*, vol. 230, pp. 193–220, 1998.

[20] M. M. Stevenson and E. M. Riley, "Innate immunity to malaria," *Nature Reviews Immunology*, vol. 4, no. 3, pp. 169–180, 2004.

[21] A. Chalifour, P. Jeannin, J. F. Gauchat et al., "Direct bacterial protein PAMP recognition by human NK cells involves TLRs and triggers α-defensin production," *Blood*, vol. 104, no. 6, pp. 1778–1783, 2004.

[22] J. C. Weissler, L. P. Nicod, M. F. Lipscomb, and G. B. Toews, "Natural killer cell function in human lung is compartmentalized," *American Review of Respiratory Disease*, vol. 135, no. 4 I, pp. 941–949, 1987.

[23] C. Grégoire, L. Chasson, C. Luci et al., "The trafficking of natural killer cells," *Immunological Reviews*, vol. 220, no. 1, pp. 169–182, 2007.

[24] T. Walzer, M. Dalod, S. H. Robbins, L. Zitvogel, and E. Vivier, "Natural-killer cells and dendritic cells: "L'union fait la force"," *Blood*, vol. 106, no. 7, pp. 2252–2258, 2005.

[25] N. Lapaque, T. Walzer, S. Méresse, E. Vivier, and J. Trowsdale, "Interactions between human NK cells and macrophages in response to Salmonella infection," *Journal of Immunology*, vol. 182, no. 7, pp. 4339–4348, 2009.

[26] F. Bellora, R. Castriconi, A. Dondero et al., "The interaction of human natural killer cells with either unpolarized or polarized macrophages results in different functional outcomes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 50, pp. 21659–21664, 2010.

[27] C. Costantini and M. A. Cassatella, "The defensive alliance between neutrophils and NK cells as a novel arm of innate immunity," *Journal of Leukocyte Biology*, vol. 89, no. 2, pp. 221–233, 2011.

[28] H. Heremans, C. Dillen, J. Van Damme, and A. Billiau, "Essential role for natural killer cells in the lethal lipopolysaccharide-induced Shwartzman-like reaction in mice," *European Journal of Immunology*, vol. 24, no. 5, pp. 1155–1160, 1994.

[29] M. Emoto, M. Miyamoto, I. Yoshizawa et al., "Critical role of NK cells rather than Vα14+NKT cells in lipopolysaccharide-induced lethal shock in mice," *Journal of Immunology*, vol. 169, no. 3, pp. 1426–1432, 2002.

[30] W. E. Carson, H. Yu, J. Dierksheide et al., "A fatal cytokine-induced systemic inflammatory response reveals a critical role for NK cells," *Journal of Immunology*, vol. 162, no. 8, pp. 4943–4951, 1999.

[31] A. R. Kerr, L. A. S. Kirkham, A. Kadioglu et al., "Identification of a detrimental role for NK cells in pneumococcal pneumonia and sepsis in immunocompromised hosts," *Microbes and Infection*, vol. 7, no. 5-6, pp. 845–852, 2005.

[32] E. R. Sherwood, V. T. Enoh, E. D. Murphey, and C. Y. Lin, "Mice depleted of CD8+ T and NK cells are resistant to injury caused by cecal ligation and puncture," *Laboratory Investigation*, vol. 84, no. 12, pp. 1655–1665, 2004.

[33] A. O. Etogo, J. Nunez, C. Y. Lin, T. E. Toliver-Kinsky, and E. R. Sherwood, "NK but not CD1-restricted NKT cells facilitate systemic inflammation during polymicrobial intra-abdominal sepsis," *Journal of Immunology*, vol. 180, no. 9, pp. 6334–6345, 2008.

[34] B. Badgwell, R. Parihar, C. Magro, J. Dierksheide, T. Russo, and W. E. Carson III, "Natural killer cells contribute to the lethality of a murine model of *Escherichi coli* infection," *Surgery*, vol. 132, no. 2, pp. 205–212, 2002.

[35] C. J. Godshall, M. J. Scott, P. T. Burch, J. C. Peyton, and W. G. Cheadle, "Natural killer cells participate in bacterial clearance during septic peritonitis through interactions with macrophages," *Shock (Augusta, Ga.)*, vol. 19, no. 2, pp. 144–149, 2003.

[36] M. J. Scott, J. J. Hoth, S. A. Gardner, J. C. Peyton, and W. G. Cheadle, "Natural killer cell activation primes macrophages to clear bacterial infection," *American Surgeon*, vol. 69, no. 8, pp. 679–686, 2003.

[37] G. Perona-Wright, K. Mohrs, F. M. Szaba et al., "Systemic but not local infections elicit immunosuppressive IL-10 production by natural killer cells," *Cell Host and Microbe*, vol. 6, no. 6, pp. 503–512, 2010.

[38] S. Kim, K. Iizuka, H. L. Aguila, I. L. Weissman, and W. M. Yokoyama, "*In vivo* natural killer cell activities revealed by natural killer cell-deficient mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 6, pp. 2731–2736, 2000.

[39] S. Kim, Y. J. Song, D. A. Higuchi et al., "Arrested natural killer cell development associated with transgene insertion into the Atf2 locus," *Blood*, vol. 107, no. 3, pp. 1024–1030, 2006.

[40] D. M. Gascoyne, E. Long, H. Veiga-Fernandes et al., "The basic leucine zipper transcription factor E4BP4 is essential for natural killer cell development," *Nature Immunology*, vol. 10, no. 10, pp. 1118–1124, 2009.

[41] M. Kashiwada, D. M. Levy, L. McKeag et al., "IL-4-induced transcription factor NFIL3/E4BP4 controls IgE class switching," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 2, pp. 821–826, 2010.

[42] T. Walzer, M. Bléry, J. Chaix et al., "Identification, activation, and selective *in vivo* ablation of mouse NK cells via NKp46," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 9, pp. 3384–3389, 2007.

[43] J. Unsinger, J. S. McDonough, L. D. Shultz, T. A. Ferguson, and R. S. Hotchkiss, "Sepsis-induced human lymphocyte apoptosis and cytokine production in "humanized" mice," *Journal of Leukocyte Biology*, vol. 86, no. 2, pp. 219–227, 2009.

[44] S. L. Zanotti-Cavazzoni, M. Guglielmi, J. E. Parrillo, T. Walker, R. P. Dellinger, and S. M. Hollenberg, "Fluid resuscitation influences cardiovascular performance and mortality in a murine model of sepsis," *Intensive Care Medicine*, vol. 35, no. 4, pp. 748–754, 2009.

[45] J. S. Orange, "Human natural killer cell deficiencies and susceptibility to infection," *Microbes and Infection*, vol. 4, no. 15, pp. 1545–1558, 2002.

[46] C. A. Biron, K. S. Byron, and J. L. Sullivan, "Severe herpesvirus infections in an adolescent without natural killer cells," *New England Journal of Medicine*, vol. 320, no. 26, pp. 1731–1735, 1989.

[47] A. Fischer, "Human primary immunodeficiency diseases," *Immunity*, vol. 27, no. 6, pp. 835–845, 2007.

[48] T. W. Kuijpers, P. A. Baars, C. Dantin, M. Van Den Burg, R. A. W. Van Lier, and E. Roosnek, "Human NK cells can control CMV infection in the absence of T cells," *Blood*, vol. 112, no. 3, pp. 914–915, 2008.

[49] R. S. Hotchkiss, K. W. Tinsley, P. E. Swanson et al., "Sepsis-induced apoptosis causes progressive profound depletion of B and CD4+ T lymphocytes in humans," *Journal of Immunology*, vol. 166, no. 11, pp. 6952–6963, 2001.

[50] E. J. Giamarellos-Bourboulis, T. Tsaganos, E. Spyridaki et al., "Early changes of CD4-positive lymphocytes and NK cells in patients with severe Gram-negative sepsis," *Critical Care*, vol. 10, no. 6, article R166, 2006.

[51] M. Holub, Z. Klučková, M. Helcl, J. Přihodov, R. Rokyta, and O. Beran, "Lymphocyte subset numbers depend on the bacterial origin of sepsis," *Clinical Microbiology and Infection*, vol. 9, no. 3, pp. 202–211, 2003.

[52] G. R. Klimpel, D. N. Herndon, and M. Fons, "Defective NK cell activity following thermal injury," *Clinical and Experimental Immunology*, vol. 66, no. 2, pp. 384–392, 1986.

[53] J. Puente, T. Carvajal, S. Parra et al., "In vitro studies of natural killer cell activity in septic shock patients. Response to a challenge with α-interferon and interleukin-2," *International Journal of Clinical Pharmacology Therapy and Toxicology*, vol. 31, no. 6, pp. 271–275, 1993.

[54] S. Zeerleder, C. E. Hack, C. Caliezi et al., "Activated cytotoxic T cells and NK cells in severe sepsis and septic shock and their role in multiple organ dysfunction," *Clinical Immunology*, vol. 116, no. 2, pp. 158–165, 2005.

[55] C. Gogos, A. Kotsaki, A. Pelekanou et al., "Early alterations of the innate and adaptive immune statuses in sepsis according to the type of underlying infection," *Critical Care*, vol. 14, article R96, 2010.

[56] F. Venet, F. Davin, C. Guignant et al., "Early assessment of leukocyte alterations at diagnosis of septic shock," *Shock*, vol. 34, pp. 358–363, 2010.

[57] C. H. Tay and R. M. Welsh, "Distinct organ-dependent mechanisms for the control of murine cytomegalovirus infection by natural killer cells," *Journal of Virology*, vol. 71, no. 1, pp. 267–275, 1997.

[58] J. M. Cavaillon and D. Annane, "Compartmentalization of the inflammatory response in sepsis and SIRS," *Journal of Endotoxin Research*, vol. 12, no. 3, pp. 151–170, 2006.

[59] C. Larroche and L. Mouthon, "Pathogenesis of hemophagocytic syndrome (HPS)," *Autoimmunity Reviews*, vol. 3, no. 2, pp. 69–75, 2004.

[60] K. Mazodier, V. Marin, D. Novick et al., "Severe imbalance of IL-18/IL-18BP in patients with secondary hemophagocytic syndrome," *Blood*, vol. 106, no. 10, pp. 3483–3489, 2005.

[61] S. E. Stepp, R. Dufourcq-Lagelouse, F. Le Deist et al., "Perforin gene defects in familial hemophagocytic lymphohistiocytosis," *Science*, vol. 286, no. 5446, pp. 1957–1959, 1999.

[62] M. B. Jordan, D. Hildeman, J. Kappler, and P. Marrack, "An animal model of hemophagocytic lymphohistiocytosis (HLH): CD8+ T cells and interferon gamma are essential for the disorder," *Blood*, vol. 104, no. 3, pp. 735–743, 2004.

[63] S. Nedvetzki, S. Sowinski, R. A. Eagle et al., "Reciprocal regulation of human natural killer cells and macrophages associated with distinct immune synapses," *Blood*, vol. 109, no. 9, pp. 3776–3785, 2007.

[64] L. Von Müller, A. Klemm, N. Durmus et al., "Cellular immunity and active human cytomegalovirus infection in patients with septic shock," *Journal of Infectious Diseases*, vol. 196, no. 9, pp. 1288–1295, 2007.

[65] L. Chiche, J. M. Forel, A. Roch et al., "Active cytomegalovirus infection is common in mechanically ventilated medical intensive care unit patients," *Critical Care Medicine*, vol. 37, no. 6, pp. 1850–1857, 2009.

[66] M. Chilet, G. Aguilar, I. Benet et al., "Virological and immunological features of active cytomegalovirus infection in nonimmunosuppressed patients in a surgical and trauma intensive care unit," *Journal of Medical Virology*, vol. 82, no. 8, pp. 1384–1391, 2010.

[67] S. C. Wesselkamper, B. L. Eppert, G. T. Motz, G. W. Lau, D. J. Hassett, and M. T. Borchers, "NKG2D is critical for NK cell activation in host defense against Pseudomonas aeruginosa respiratory infection," *Journal of Immunology*, vol. 181, no. 8, pp. 5481–5489, 2008.

[68] Y. T. Bryceson, C. Fauriat, J. M. Nunes et al., "Functional analysis of human NK cells by flow cytometry," *Methods in Molecular Biology*, vol. 612, pp. 335–352, 2010.

[69] T. Walzer, S. Jaeger, J. Chaix, and E. Vivier, "Natural killer cells: from CD3(-)NKp46(+) to post-genomics meta-analyses," *Current Opinion in Immunology*, vol. 19, no. 3, pp. 365–372, 2007.

[70] D. Chaussabel, C. Quinn, J. Shen et al., "A modular analysis framework for blood genomics studies: application to systemic lupus erythematosus," *Immunity*, vol. 29, no. 1, pp. 150–164, 2008.

[71] M. P. R. Berry, C. M. Graham, F. W. McNab et al., "An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis," *Nature*, vol. 466, no. 7309, pp. 973–977, 2010.

[72] M. Terme, E. Ullrich, N. F. Delahaye, N. Chaput, and L. Zitvogel, "Natural killer cell-directed therapies: moving from unexpected results to successful strategies," *Nature Immunology*, vol. 9, no. 5, pp. 486–494, 2008.

[73] S. Inoue, J. Unsinger, C. G. Davis et al., "IL-15 prevents apoptosis, reverses innate and adaptive immune dysfunction, and improves survival in sepsis," *Journal of Immunology*, vol. 184, no. 3, pp. 1401–1409, 2010.

[74] W. D. Döcke, F. Randow, U. Syrbe et al., "Monocyte deactivation in septic patients: restoration by IFN-γ treatment," *Nature Medicine*, vol. 3, no. 6, pp. 678–681, 1997.

[75] R. A. Lukaszewski, A. M. Yates, M. C. Jackson et al., "Presymptomatic prediction of sepsis in intensive care unit patients," *Clinical and Vaccine Immunology*, vol. 15, no. 7, pp. 1089–1094, 2008.

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Review Article

Clinical Cancer Therapy by NK Cells via Antibody-Dependent Cell-Mediated Cytotoxicity

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Natural killer (NK) cells are powerful effector cells that can be directed to eliminate tumor cells through tumor-targeted monoclonal antibodies (mAbs). Some tumor-targeted mAbs have been successfully applied in the clinic and are included in the standard of care for certain malignancies. Strategies to augment the antitumor response by NK cells have led to an increased understanding of how to improve their effector responses. Next-generation reagents, such as molecularly modified mAbs and mAb-cytokine fusion proteins (immunocytokines, ICs) designed to augment NK-mediated killing, are showing promise in preclinical and some clinical settings. Continued research into the antitumor effects induced by NK cells and tumor-targeted mAbs suggests that additional intrinsic and extrinsic factors may influence the antitumor response. Therefore more research is needed that focuses on evaluating which NK cell and tumor criteria are best predictive of a clinical response and which combination immunotherapy regimens to pursue for distinct clinical settings.

1. Introduction

Natural killer (NK) cells are innate immune effector cells capable of recognizing and destroying virally infected and neoplastic cells. The importance of NK cell-mediated immunosurveillance in the control of tumor growth has been evaluated in NK cell-deficient mouse models with limited information in humans. Humans with NK cell deficiencies are plagued with persistent acute viral infections, especially herpes simplex virus [1]. However, mouse models with defects in NK cell effector function clearly demonstrate an increased susceptibility to neoplastic disease as they age [2].

NK cell effector functions can be exploited for the treatment of some tumors through their ability to mediate antibody-dependent cellular cytotoxicity (ADCC). The NK cell Fc receptor, CD16 (FcγRIIIa), contains an immunotyrosine-activating motif (ITAM) in the cytoplasmic domain. NK cell recognition of an antibody-coated target cell results in rapid NK cell activation and degranulation [3]. mAbs that specifically target tumor cells take advantage of the ADCC effector pathway to tip the balance of an interrogating NK cell in the favor of the activating receptors resulting in tumor cell destruction and an anti-tumor immune response [4].

Tumor-targeted mAbs that initiate NK cell ADCC have been used clinically. Antibodies targeting CD20, Her2/neu, epidermal growth factor receptor (EGFR), and disaloganglioside (GD2) are examples of clinically successful antibodies whose mechanisms include NK cell-mediated ADCC [5–9]. GD2 is overexpressed on tumors of neuroectodermal origin, such as neuroblastoma and melanoma, and minimally expressed in normal tissues making it a good target for tumor-specific mAb. Anti-GD2 mAbs work through NK cell-mediated ADCC and have demonstrated clinical benefit for children with neuroblastoma [10]. In this paper, we will use examples from mAbs targeting GD2 and other tumor antigens to discuss antibody-facilitated NK cell-mediated cancer immunotherapy strategies.

2. NK Cell Responses to Tumor-Specific mAbs

There are numerous Fc receptors for IgG (FcγR) that are widely expressed on immune cells. The FcγR family consists of four classes of receptors, FcγRI, FcγRII, FcγRIII, and FcγRIV, that have been identified in both mice and humans. There are significant similarities in the functions of the FcγR receptors between mice and humans, but there is limited homology in receptors themselves [11]. To date, only one inhibitory FcγR, FcγRIIb, has been identified and is the only receptor to have complete homology between mice and humans [11]. FcγRs can be found on virtually all hematopoietic cells except T cells; in most cases, cells coexpress activating and inhibitory FcγR, allowing for the balance between activating and inhibitory receptors to dictate their response [11]. NK cells are an exception to this rule and express only the activating FcγRIIIa. NK cells do not express the inhibitory FcγRIIb.

Because of their rapid and unopposed responses to mAb, NK cells play a major role in the anti-tumor response elicited by tumor-specific mAbs. Early studies demonstrated that NK cells are the primary mediators of the immune response elicited by tumor-targeted mAbs. However, recent data show that granulocytes may also play a significant role in anti-tumor responses generated by tumor-targeted antibodies [11, 12]. NK cells are important effectors in the mAb-driven immune response to tumors, and data continue to accumulate on their importance [13]. Multiple clinically successful mAbs utilize NK-mediated ADCC as a mechanism of action. Rituximab (anti-CD20), Herceptin (anti-Her-2/neu), Cetuximab (anti-EGFR), and the anti-GD2-mAbs 3F8 and ch14.18 are examples of tumor-specific mAbs whose clinical activity can be attributed, at least in part, to NK cells.

3. Augmentation of mAb Responses through the Activation of NK Cells

Various strategies of NK cell activation alongside antibody administration have been evaluated. NK cell activation can occur through a variety of stimuli including cytokine administration, toll-like receptor (TLR) agonists, or agonist antibodies directed toward activating receptors on NK cells. The coadministration of an NK-activating cytokine, interleukin-2 (IL-2), enhances the anti-tumor activity of NK cells [14, 15]. The TLR9 agonist CpG can activate numerous innate immune effectors, including NK cells [16]. Combination of CpG with Rituximab increases NK-mediated ADCC in vitro and anti-tumor responses in a mouse model of CD20-expressing tumors [17].

Further activation of NK cells through numerous receptors including 4-1BB or the Fc receptor FcγRIIIa increases ADCC activity [18]. Antibodies with Fc regions that have higher affinity for FcγRIIIa are better at activating NK cells while simultaneously initiating additional NK effector pathways [19]. High-affinity Fc-antibodies can be used at lower concentrations than traditional antibodies and maintain anti-tumor activity [19]. Antibodies with higher affinity for FcγRIIIa may be beneficial in a clinical setting by reducing the amount of antibody necessary to produce an antitumor response and therefore reduce mAb-related toxicities [20]. 4-1BB (CD137) is an activating receptor on the surface of NK cells [21]. The activation of NK cells with an agonistic antibody to 4-1BB has recently been described in a mouse model of B-cell lymphoma [18]. 4-1BB is increased on $CD56^{dim}$ effector NK cells after CD16-mediated activation. Activation of NK cells with a 4-1BB agonist antibody between Rituximab courses in vivo led to complete regression of subcutaneous murine lymphoma tumors by NK cells [18].

The combination of mAb therapy with cytokines is another strategy used to increase their activity. Combination of Herceptin with interleukin-12 (IL-12), an important cytokine to NK cell responsiveness and IFNγ production, increases the response of NK cells to Her2-expressing breast tumor cells in a mouse model of breast cancer [22]. Clinical development of this concept is underway [23]. Augmentation of NK cell responses by the addition of exogenous IL-2 has been extensively demonstrated to increase the anti-tumor response of antibody therapy. IL-2-activated lymphokine-activated killer (LAK) cells have increased ADCC activity against mAb-coated tumor cells [14, 15, 24]. IL-2 lowers the required amount of antibody necessary for NK cells to effectively lyse antibody-coated tumor targets [25]. Increased NK cell effector function after IL-2 activation is true of NK cells isolated from humans, mice, and dogs [26, 27].

4. Altered mAbs That Increase NK Cell Effector Functions

Following the production of the initial 14.18 murine anti-GD2 mAb, several molecular modifications have resulted in 2nd and 3rd generation reagents, designed to have improved function. First, there was the class switch to murine IgG2a to augment ADCC (creating the 14.G2a mAb). This was followed the by creation of a chimeric antibody (ch14.18), a humanized antibody (hu14.18), and multiple altered hu14.18 antibodies to enhance the anti-tumor response [10]. Humanized anti-GD2 mAb hu14.18K322A (K322A) is a new-generation anti-GD2 mAb that has been designed to stimulate NK cell effector mechanisms and simultaneously reduce some of the toxicities associated with anti-GD2 therapy [5]. hu14.18K322A has two key differences from its hu14.18 parent. First, hu14.18K322A was produced in a rat hybridoma line, YB2/0. YB2/0 cells have low fucosyltrasferase activity and therefore produce antibodies with fewer fucose side chains on the Fc portion. IgG antibodies that have low or absent fucose side chains are more effective at eliciting ADCC [28, 29]. Second, hu14.18K322A has a point mutation at the 322 position resulting in the replacement of lysine 322 with an alanine. This specific mutation reduces the ability of hu14.18K322A to activate complement compared to its anti-GD2 relatives [30]. Allodynia, the major clinical toxicity associated with anti-GD2 therapy, is likely the result of complement fixation. Therefore, hu14.18K322A is designed to retain or potentially enhance NK-mediated anti-tumor responses while reducing the antibody's toxicity [30].

Immunocytokines (ICs) are antibodies with linked cytokines at the Fc terminal end. The anti-GD2 IC hu14.18-IL-2 is a humanized mAb with two functional interleukin-2 proteins at the Fc terminal end [31, 32]. ICs may have certain advantages over traditional mAbs [33]. In several preclinical models, using 3 different ICs, the IC provided far greater antitumor effects than the same amount of the naked mAb infused with the same amount of IL2 (but infused simultaneously as separate molecules rather than as the IC fusion protein). This may be because ICs transport cytokine to the site of tumor and can support an ongoing local anti-tumor immune response [34, 35]. Direct delivery of IC into the tumor itself elicits a more potent local effect. Intratumoral injection of IC in tumor-bearing mice induces better antitumor responses than systemic administration. This effect can be attributed to its activating effects on intratumoral NK cells [34].

One advantage of using IC is its effect on the formation of an immune synapse between the Ab-coated tumor cell and the NK cell. Recent data from our laboratory suggest that NK recognition of an IC involves not only the Fc receptor, but also IL2 receptors [36, 37]. The involvement of the IL2R increases IC-facilitated conjugate formation between NK cells and tumor cells (Figure 1) [36, 37]. Furthermore, IC may facilitate NK: tumor cell conjugation in the absence of Fc receptors. Using an NK cell line with minimal, if any, expression of FcγRIIIa, we recently demonstrated that the IL2Rα chain plays an important role in NK: tumor cell conjugation [36]. The association of NK cells with IC-coated tumor cell results in the formation of an activated immune synapse (AIS), defined by the localization of LFA-1and CD2 [37]. AIS formation facilitated by an IC is hallmarked by clustering of NK cell CD25 into the synapse and can also be abrogated by CD25 blockade [37]. The potential benefit of IL-2 containing ICs in activating and assisting NK cells in tumor cell destruction is a relatively new research area for clinical NK-mediated tumor immunotherapy.

5. Recent Clinical Results with Anti-GD2 mAb-Induced ADCC

High-risk childhood neuroblastoma remains a disease that has not shown major improvements in cure rates over the past 2 decades [38]. Preclinical and early clinical work suggested that anti-GD2-based mAb therapy would be most effective if given in the setting of minimal residual disease, and in combination with cytokines that augment ADCC. A large randomized study was conducted by the Children's Oncology Group (COG) to test these concepts. Following initial response to combined agent chemotherapy, surgery, ablative chemotherapy, and autologous hematopoietic stem cell transplant, children received isotretinoin and were randomized to receive immunotherapy (the ch14.18 chimeric mAb + IL2 + GM-CSF) or no immunotherapy. Two-hundred twenty-six children were randomized, and the group receiving immunotherapy showed a 2-year event-free survival of 66% versus 46% for the no-immunotherapy group ($P = .012$) [39]. In the USA, through the COG, this immunotherapy regimen has thus become the "standard of care" maintenance treatment for high-risk patients that have responded to their initial therapy.

The next-generation immunotherapy approach has involved the hu14.18-IL2 IC. Our hypothesis, based on our preclinical data [27], was that the IC approach would work best for children with smaller amounts of refractory/relapsed neuroblastoma. A recent phase II COG study in children with relapsed or refractory neuroblastoma showed that 7 of 24 patients with "nonbulky" disease showed evidence of antitumor activity, while 0 of 13 with bulky disease had evidence of antitumor activity [40]. The results of this study are consistent with the hypothesis of better activity for nonbulky disease ($P = .03$). We are continuing to develop this agent and are hoping that a future large COG trial will enable us to test this genetically engineered molecule for children in remission in order to prevent relapse, as was done for the separate ch14.18 mAb with GM-CSF and IL2 regimen [39].

6. Fc Receptor Polymorphisms and NK Responses to mAbs and ICs

Two allelic polymorphisms have been identified in human FcγRIIIa at position 158. The aa at this location in the receptor interacts with the hinge region of IgG antibodies and affects the magnitude of response at subsaturating concentrations of IgG [41]. The one aa difference of either a phenylalanine (f) or a valine (v) in FcγRIIIa may have implications for mAb therapy [42]. NK cells containing a valine at position 158 have a higher affinity for IgG. NK cells isolated from individuals bearing this receptor contain more cytophilic IgG when examined directly ex vivo [42]. FcγRIIIa^{158v} is associated with a less favorable prognosis for autoimmune sufferers [43]. FcγRIIIa^{158v} NK cells are more sensitive to activation and have a higher calcium influx and more rapid induction of activation-induced cell death (AICD) when stimulated [44]. A more sensitive activating receptor that produces a stronger intracellular response, such as Ca^{2+} influx, may support a more productive immune synapse by localizing granules faster to the centrosome, as has been shown with T-cell responses to TCR stimulation [45]. Faster granule localization results in more granules loaded into the synapse for target cell destruction [45]. Therefore, NK cells with FcγRIIIa^{158v} receptors may have a twofold advantage in the setting of mAb-mediated cancer immunotherapy: (1) enhanced ability to recognize and bind to tumor cells coated with mAb molecules and (2) the release of more granules for each tumor cell they encounter.

Patients with an FcγRIIIa^{158v} genotype respond better to therapy that utilizes an ADCC-mediating mAb as a mechanism of action. Studies using patient samples during treatment with Herceptin, Rituximab, and Cetuximab have correlated an FcγRIIIa^{158v} receptor genotype with better response to therapy [46–48]. The response of FcγRIIIa^{158v} NK cells in an in vitro assay of ADCC against antibody-coated tumor cells can be used as a predictor for patient response to therapy [49]. The role of Fc receptor polymorphisms on the response to immunocytokine in comparison

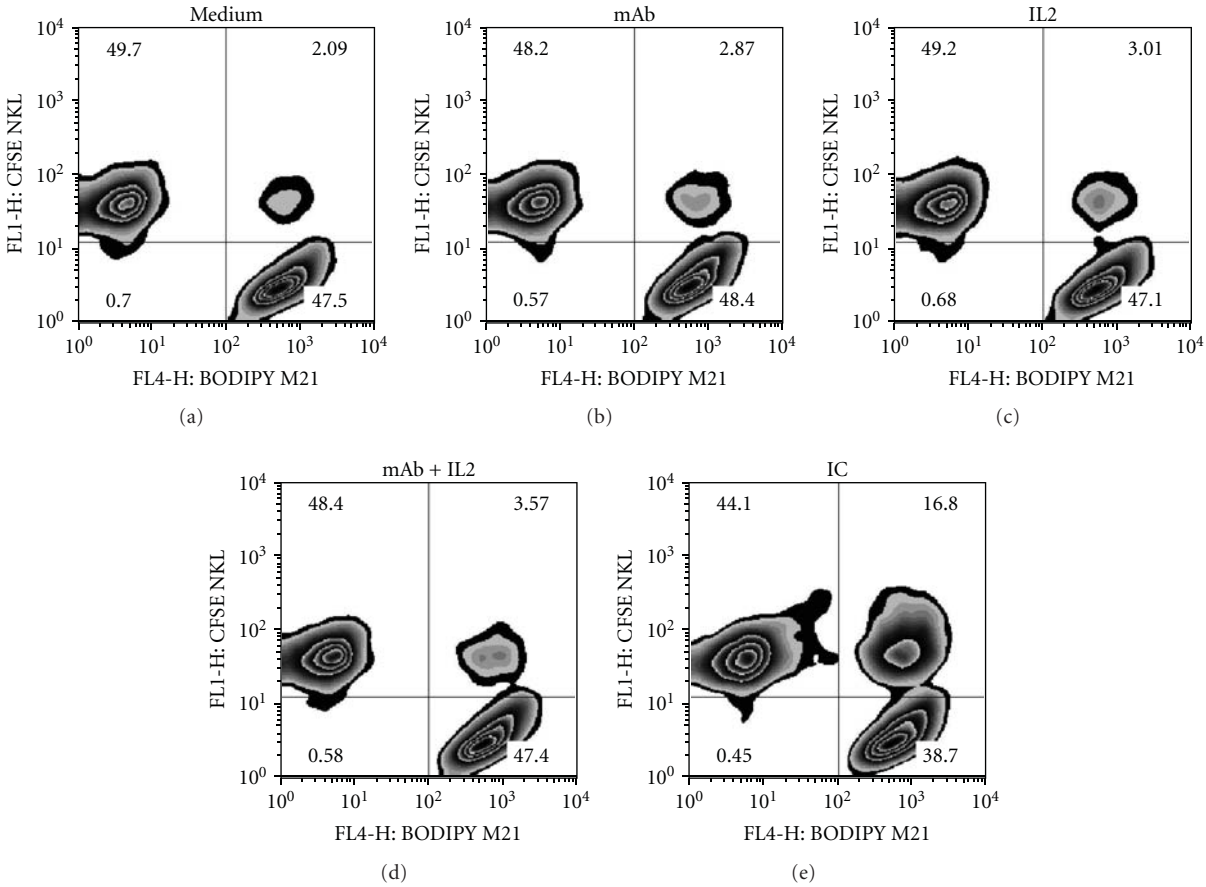

Figure 1: Immunocytokine facilitates adhesion of NK cells to tumor cells. This figure, adapted from Buhtoiarov et al. [36], shows the adhesion of M21 human melanoma tumor cells to NKL human NK cells after coincubation for 30 minutes. Tumor cells were prelabeled with CFSE, and NK cells were prelabeled with BODIPY and coincubated in medium alone, with hu14.18 (mAb) alone, soluble IL2, hu14.18 + soluble IL2, or hu14.18-IL2 immunocytokine (IC). The percentage of cells that had formed conjugates was defined as double positive cells as determined by flow cytometry.

with mAb remains to be answered. A recent study from our laboratory [12] evaluated patient samples from our COG phase II anti-GD2 IC trial for FcγRIIIa genotypes [40]. This study only had two patients with an FcγRIIIa$^{158v/v}$ genotype and was therefore inconclusive. The identification of FcγR polymorphisms that affect clinical responses to mAb therapy may suggest a new criterion for more tailored patient selection for mAb therapy. The importance of FcγRIIIa polymorphisms in IC-induced anti-tumor responses will be of considerable interest because of the involvement of the IL2R.

7. KIRs and the Response to mAbs and ICs

An important factor in NK-mediated therapy is the intrinsic ability for NK cells to respond to stimuli. The licensing hypothesis of NK cell function was developed after the observation that NK cells from mice lacking MHC-I, an important NK inhibitory ligand, respond poorly [50]. The intricate system of killer cell immunoglobulin-like receptors (KIRs) in humans and Ly-49 receptors in mice recognizes normally expressed MHC-I antigens on neighboring cells and inhibit NK cell effector functions [51]. According to the licensing hypothesis, an NK cell that does not encounter a ligand for one of its KIR or Ly49 inhibitory receptors during development is functionally deficient [50]. However, KIR and Ly49 genes are inherited on different chromosomes from MHC, and therefore many individuals and mouse strains have at least one KIR or Ly49 that lacks a corresponding ligand [51]. These individuals and mouse strains have at least one population of functionally deficient NK cells [50], reflecting the NK population that contains the KIR receptor corresponding to the KIR/KIR-L mismatch. Clinical studies evaluating KIR/KIR-L matching have estimated that ~60% of people have at least one KIR for which they lack a corresponding receptor and are therefore KIR/KIR-L mismatched for at least one locus [51].

Some preclinical studies have challenged the importance of licensing in certain models. Orr et al. evaluated the response of licensed versus unlicensed NK cells in response to murine cytomegalovirus (MCMV). C57Bl/6 mice have a "licensed" subset of NK cells expressing Ly49C/I, the receptor for H2^b and "unlicensed" NK cells expressing an

activating Ly49H. After infection of B6 mice with MCMV, a virus for which NK cell function is necessary for control, LY49C/I^{neg} Ly49H^{+} ("unlicensed") NK cells were able to respond to infection [52]. This paper elegantly demonstrated that unlicensed NK cells still maintain some functionality to activating signals, although their effector responses are still less powerful than that of licensed NK cells.

New data suggest that "licensing" may not be a process restricted to NK cell development but is actually a continuous process that even mature NK cells use to respond to their environment [53, 54]. Recently, two separate reports compared adoptive transfer studies between wild type (WT) and β2m knockout (MHC-I deficient) mice to show that mature NK cells become hyporesponsive when they are put into an environment lacking MHC and vice versa [53, 54]. In these studies, only NK cells expressing an inhibitory Ly49 for which there was a cognate MHC-I ligand present could respond in the new surroundings. These studies suggest that "licensing" is a continuous (rather than absolute) mechanism that NK cells use to judge and appropriately respond to a changing environment.

While studies using murine models have been valuable in dissecting the potential factors affecting NK cell responsiveness, some observations of better anti-tumor responses by patients that are self-KIR/KIR-L mismatched have been made at multiple institutions [12, 55, 56]. Two studies evaluated anti-tumor responses in pediatric cancer patients following autologous stem cell transplant [55, 56]. These two independent studies both observed an association between disease-free survival and autologous KIR/KIR-L mismatch. Our laboratory recently evaluated the KIR/KIR-L status [12] of neuroblastoma patient samples from our COG phase II study of the anti-GD2-IC, hu14.18-IL2 [40]. In this study, we observed a better response to IC in patients that were KIR/KIR-L mismatched. These data suggest that the "unlicensed" NK cells in these patients were still involved in mediating the observed antitumor effect after IC treatment, consistent with some retained NK function by "unlicenced" NK cells. Previous reports have suggested that KIR-deficient NK cells have impaired responses to CD16-mediated stimulation [57]. In this study using NK cells from healthy donors, fewer KIR-deficient CD56^{dim} NK cells produced IFNγ or upregulated CD107a in response to either antibody-coated tumor cells or plate bound anti-CD16. However, our study using patient samples [12] and evaluating response to IC rather than naked mAb warrants a closer examination of NK cell "licensing" in the context of IC-mediated ADCC.

8. Conclusions

In the relatively short time since the first description of NK cells, just over 35 years ago [58], we have learned a great deal about their function. This knowledge has allowed us to design therapeutic strategies that utilize the powerful effector mechanisms of NK cells for multiple malignancies [6–8, 40]. Our understanding of NK cells, NK cell effector responses, and the signals that drive them, continues to expand. NK cells are capable of eliminating tumor cells coated with an IgG antibody in vitro and in some patients, and activation of NK cells with cytokines such as IL2 increases the anti-tumor effect [14, 15, 24, 46]. This approach has led to successful therapeutic mAbs for certain tumors but still do not elicit a response in all patients [38].

Our continued understanding of the factors that affect the response to Ab-coated tumor cells in the tumor environment is important for the creation of the next-generation mAbs and therapeutic strategies. Important intrinsic factors that affect NK cell responsiveness to Ab-coated tumor cells include the expressed variants of FcγRIIIa and the intrinsic ability of individual NK cell subsets to respond to stimuli due to their "licensing" status [12, 50]. Separate factors include the mass of tumor present when immunotherapy is given, the activation state of the effector cells mediating ADCC, and potential other receptor-ligand interactions that influence the synapse formed between NK and tumor cells. Next-generation immunocytokines may have a functional advantage over traditional mAbs in activation of certain NK cell subsets because of the contribution of cytokine receptors [33, 36, 37].

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References

[1] J. S. Orange, "Human natural killer cell deficiencies and susceptibility to infection," *Microbes and Infection*, vol. 4, no. 15, pp. 1545–1558, 2002.

[2] J. A. Trapani and I. Voskoboinik, "Infective, neoplastic, and homeostatic sequelae of the loss of perforin function in humans," *Advances in experimental medicine and biology*, vol. 601, pp. 235–242, 2007.

[3] J. V. Ravetch and S. Bolland, "IgG Fc receptors," *Annual Review of Immunology*, vol. 19, pp. 275–290, 2001.

[4] P. M. Sondel and J. A. Hank, "Antibody-directed, effector cell-mediated tumor destruction," *Hematology/Oncology Clinics of North America*, vol. 15, no. 4, pp. 703–721, 2001.

[5] F. Navid, V. M. Santana, and R. C. Barfield, "Anti-GD2 antibody therapy for GD2-expressing tumors," *Current Cancer Drug Targets*, vol. 10, no. 2, pp. 200–209, 2010.

[6] K. P. Garnock-Jones, G. M. Keating, and L. J. Scott, "Trastuzumab: a review of its use as adjuvant treatment in human epidermal growth factor receptor 2 (HER2)-positive early breast cancer," *Drugs*, vol. 70, no. 2, pp. 215–239, 2010.

[7] J. García-Foncillas and E. Díaz-Rubio, "Progress in metastatic colorectal cancer: growing role of cetuximab to optimize clinical outcome," *Clinical and Translational Oncology*, vol. 12, no. 8, pp. 533–542, 2010.

[8] M. C. Winter and B. W. Hancock, "Ten years of rituximab in NHL," *Expert Opinion on Drug Safety*, vol. 8, no. 2, pp. 223–235, 2009.

[9] M. R. Albertini, J. A. Hank, and P. M. Sondel, "Native and genetically engineered anti-disialoganglioside monoclonal antibody treatment of melanoma," *Cancer Chemotherapy and Biological Response Modifiers*, vol. 22, pp. 789–797, 2005.

[10] R. K. Yang and P. M. Sondel, "Anti-GD2 strategy in the treatment of neuroblastoma," *Drugs Future*, vol. 35, no. 8, p. 665, 2010.

[11] F. Nimmerjahn and J. V. Ravetch, "Fcγ receptors as regulators of immune responses," *Nature Reviews Immunology*, vol. 8, no. 1, pp. 34–47, 2008.

[12] D. C. Delgado, J. A. Hank, J. Kolesar et al., "Genotypes of NK cell KIR receptors, their ligands, and Fcγ receptors in the response of neuroblastoma patients to Hu14.18-IL2 immunotherapy," *Cancer Research*, vol. 70, no. 23, pp. 9554–9561, 2010.

[13] A. Beano, E. Signorino, A. Evangelista et al., "Correlation between NK function and response to trastuzumab in metastatic breast cancer patients," *Journal of Translational Medicine*, vol. 6, article no. 25, 2008.

[14] J. A. Hank, G. Weil-Hillman, J. E. Surfus, J. A. Sosman, and P. M. Sondel, "Addition of interleukin-2 in vitro augments detection of lymphokine-activated killer activity generated in vivo," *Cancer Immunology Immunotherapy*, vol. 31, no. 1, pp. 53–59, 1990.

[15] J. R. Ortaldo, C. Woodhouse, and A. C. Morgan, "Analysis of effector cells in human antibody-dependent cellular cytotoxicity with murine monoclonal antibodies," *Journal of Immunology*, vol. 138, no. 10, pp. 3566–3572, 1987.

[16] H. H. Van Ojik, L. Bevaart, C. E. Dahle et al., "CpG-A and B oligodeoxynucleotides enhance the efficacy of antibody therapy by activating different effector cell populations," *Cancer Research*, vol. 63, no. 17, pp. 5595–5600, 2003.

[17] D. J. Betting, R. E. Yamada, K. Kafi, J. Said, N. Van Rooijen, and J. M. Timmerman, "Intratumoral but not systemic delivery of CpG oligodeoxynucleotide augments the efficacy of anti-CD20 monoclonal antibody therapy against B Cell lymphoma," *Journal of Immunotherapy*, vol. 32, no. 6, pp. 622–631, 2009.

[18] H. E. Kohrt, R. Houot, M. J. Goldstein et al., "CD137 stimulation enhances the antilymphoma activity of anti-CD20 antibodies," *Blood*, vol. 117, no. 8, pp. 2423–2432, 2011.

[19] J. A. Bowles, S. Y. Wang, B. K. Link et al., "Anti-CD20 monoclonal antibody with enhanced affinity for CD16 activates NK cells at lower concentrations and more effectively than rituximab," *Blood*, vol. 108, no. 8, pp. 2648–2654, 2006.

[20] L. M. Weiner, R. Surana, and S. Wang, "Monoclonal antibodies: versatile platforms for cancer immunotherapy," *Nature Reviews Immunology*, vol. 10, no. 5, pp. 317–327, 2010.

[21] W. Lin, C. J. Voskens, X. Zhang et al., "Fc-dependent expression of CD137 on human NK cells: insights into "agonistic" effects of anti-CD137 monoclonal antibodies," *Blood*, vol. 112, no. 3, pp. 699–707, 2008.

[22] R. Parihar, J. Dierksheide, Y. Hu, and W. E. Carson, "IL-12 enhances the natural killer cell cytokine response to Ab-coated tumor cells," *Journal of Clinical Investigation*, vol. 110, no. 7, pp. 983–992, 2002.

[23] R. Parihar, P. Nadella, A. Lewis et al., "A phase I study of interleukin 12 with trastuzumab in patients with human epidermal growth factor receptor-2-overexpressing malignancies: analysis of sustained interferon γ production in a subset of patients," *Clinical Cancer Research*, vol. 10, no. 15, pp. 5027–5037, 2004.

[24] K. R. Schultz, J. P. Klarnet, D. J. Peace et al., "Monoclonal antibody therapy of murine lymphoma: enhanced efficacy by concurrent administration of interleukin 2 or lymphokine-activated killer cells," *Cancer Research*, vol. 50, no. 17, pp. 5421–5425, 1990.

[25] M. Watanabe, T. Kubota, M. Kitajima, and S. Hakomori, "Synergetic effect of interleukin-2 and cellular cytotoxicity against a novel tumor-associated carbohydrate antigen Le(a)/Le(a) (dimeric Le(a) mediated by monoclonal antibody NCC-ST-421 in adoptive immunization using SCID mice," *Cancer Immunology Immunotherapy*, vol. 37, no. 4, pp. 245–250, 1993.

[26] S. C. Helfand, S. A. Soergel, R. L. Donner et al., "Potential to involve multiple effector cells with human recombinant interleukin-2 and antiganglioside monoclonal antibodies in a canine malignant melanoma immunotherapy model," *Journal of Immunotherapy*, vol. 16, no. 3, pp. 188–197, 1994.

[27] Z. C. Neal, J. C. Yang, A. L. Rakhmilevich et al., "Enhanced activity of hu14.18-IL2 immunocytokine against murine NXS2 neuroblastoma when combined with interleukin 2 therapy," *Clinical Cancer Research*, vol. 10, no. 14, pp. 4839–4847, 2004.

[28] S. Iida, R. Kuni-Kamochi, K. Mori et al., "Two mechanisms of the enhanced antibody-dependent cellular cytotoxicity (ADCC) efficacy of non-fucosylated therapeutic antibodies in human blood," *BMC Cancer*, vol. 9, article no. 58, 2009.

[29] T. Shinkawa, K. Nakamura, N. Yamane et al., "The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity," *Journal of Biological Chemistry*, vol. 278, no. 5, pp. 3466–3473, 2003.

[30] L. S. Sorkin, M. Otto, W. M. Baldwin et al., "Anti-GD with an FC point mutation reduces complement fixation and decreases antibody-induced allodynia," *Pain*, vol. 149, no. 1, pp. 135–142, 2010.

[31] S. D. Gillies, D. Young, K. M. Lo, and S. Roberts, "Biological activity and in vivo clearance of antitumor antibody/cytokine fusion proteins," *Bioconjugate Chemistry*, vol. 4, no. 3, pp. 230–235, 1993.

[32] J. A. Hank, J. E. Surfus, J. Gan et al., "Activation of human effector cells by a tumor reactive recombinant anti-ganglioside GD interleukin-2 fusion protein (ch14.18-IL2)," *Clinical Cancer Research*, vol. 2, no. 12, pp. 1951–1959, 1996.

[33] B. H. Yamane, J. A. Hank, M. R. Albertini, and P. M. Sondel, "The development of antibody-IL-2 based immunotherapy with hu14.18-IL2 (EMD-273063) in melanoma and neuroblastoma," *Expert Opinion on Investigational Drugs*, vol. 18, no. 7, pp. 991–1000, 2009.

[34] E. E. Johnson, H. D. Lum, A. L. Rakhmilevich et al., "Intratumoral immunocytokine treatment results in enhanced antitumor effects," *Cancer Immunology, Immunotherapy*, vol. 57, no. 12, pp. 1891–1902, 2008.

[35] H. N. Lode, R. Xiang, N. M. Varki, C. S. Dolman, S. D. Gillies, and R. A. Reisfeld, "Targeted interleukin-2 therapy for spontaneous neuroblastoma metastases to bone marrow," *Journal of the National Cancer Institute*, vol. 89, no. 21, pp. 1586–1594, 1997.

[36] I. N. Buhtoiarov, Z. C. Neal, J. Gan et al., "Differential internalization of hu14.18-IL2 immunocytokine by NK and tumor cell: impact on conjugation, cytotoxicity, and targeting," *Journal of Leukocyte Biology*, vol. 89, no. 4, pp. 625–638, 2011.

[37] J. A. A. Gubbels, B. Gadbaw, I. N. Buhtoiarov et al., "Ab-IL2 fusion protein mediate NK cell immune synapse formation by polarizing CD25 to the target cell-effector cell interface," *Cancer Immunology and Immunotherapy*. Manuscript Submitted.

[38] J. M. Maris, "Recent advances in neuroblastoma," *New England Journal of Medicine*, vol. 362, no. 23, pp. 2154–2211, 2010.

[39] A. L. Yu, A. L. Gilman, M. F. Ozkaynak et al., "Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma," *New England Journal of Medicine*, vol. 363, no. 14, pp. 1324–1334, 2010.

[40] S. Shusterman, W. B. London, S. D. Gillies et al., "Antitumor activity of Hu14.18-IL2 in patients with relapsed/refractory neuroblastoma: a Children's Oncology Group (COG) phase II study," *Journal of Clinical Oncology*, vol. 28, no. 33, pp. 4969–4975, 2010.

[41] S. Dall'Ozzo, S. Tartas, G. Paintaud et al., "Rituximab-dependent cytotoxicity by natural killer cells: influence of FCGR3A polymorphism on the concentration-effect relationship," *Cancer Research*, vol. 64, no. 13, pp. 4664–4669, 2004.

[42] H. R. Koene, M. Kleijer, J. Algra, D. Roos, A. E. G. KR. Von Dem Borne, and M. De Haas, "FcγRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell FCγRIIIa, independently of the FCγRIIIa-48L/R/H phenotype," *Blood*, vol. 90, no. 3, pp. 1109–1114, 1997.

[43] H. M. Dijstelbloem, R. H. M. Scheepers, W. W. Oost et al., "Fcγ receptor polymorphisms in Wegener's granulomatosis: risk factors for disease relapse," *Arthritis and Rheumatism*, vol. 42, no. 9, pp. 1823–1827, 1999.

[44] J. Wu, J. C. Edberg, P. B. Redecha et al., "A novel polymorphism of FcγRIIIa (CD16) alters receptor function and predisposes to autoimmune disease," *Journal of Clinical Investigation*, vol. 100, no. 5, pp. 1059–1070, 1997.

[45] M. R. Jenkins, A. Tsun, J. C. Stinchcombe, and G. M. Griffiths, "The strength of T cell receptor signal controls the polarization of cytotoxic machinery to the immunological synapse," *Immunity*, vol. 31, no. 4, pp. 621–631, 2009.

[46] G. Cartron, L. Dacheux, G. Salles et al., "Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcγrIIIa gene," *Blood*, vol. 99, no. 3, pp. 754–758, 2002.

[47] A. Musolino, N. Naldi, B. Bortesi et al., "Immunoglobulin g fragment c receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer," *Journal of Clinical Oncology*, vol. 26, no. 11, pp. 1789–1796, 2008.

[48] WU. Zhang, M. Gordon, A. M. Schultheis et al., "FCGR2A and FCGR3A polymorphisms associated with clinical outcome of epidermal growth factor receptor-expressing metastatic colorectal cancer patients treated with single-agent cetuximab," *Journal of Clinical Oncology*, vol. 25, no. 24, pp. 3712–3718, 2007.

[49] W. K. Weng and R. Levy, "Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma," *Journal of Clinical Oncology*, vol. 21, no. 21, pp. 3940–3947, 2003.

[50] A. H. Jonsson and W. M. Yokoyama, "Natural killer cell tolerance licensing and other mechanisms," *Advances in Immunology*, vol. 101, pp. 27–79, 2009.

[51] C. Vilches and P. Parham, "KIR: diverse, rapidly evolving receptors of innate and adaptive immunity," *Annual Review of Immunology*, vol. 20, pp. 217–251, 2002.

[52] M. T. Orr, W. J. Murphy, and L. L. Lanier, "'Unlicensed' natural killer cells dominate the response to cytomegalovirus infection," *Nature Immunology*, vol. 11, no. 4, pp. 321–327, 2010.

[53] J. M. Elliott, J. A. Wahle, and W. M. Yokoyama, "MHC class I-deficient natural killer cells acquire a licensed phenotype after transfer into an MHC class I-sufficient environment," *Journal of Experimental Medicine*, vol. 207, no. 10, pp. 2073–2079, 2010.

[54] N. T. Joncker, N. Shifrin, F. Delebecque, and D. H. Raulet, "Mature natural killer cells reset their responsiveness when exposed to an altered MHC environment," *Journal of Experimental Medicine*, vol. 207, no. 10, pp. 2065–2072, 2010.

[55] W. Leung, R. Handgretinger, R. Iyengar, V. Turner, M. S. Holladay, and G. A. Hale, "Inhibitory KIR-HLA receptor-ligand mismatch in autologous haematopoietic stem cell transplantation for solid tumour and lymphoma," *British Journal of Cancer*, vol. 97, no. 4, pp. 539–542, 2007.

[56] J. M. Venstrom, J. Zheng, N. Noor et al., "KIR and HLA genotypes are associated with disease progression and survival following autologous hematopoietic stem cell transplantation for high-risk neuroblastoma," *Clinical Cancer Research*, vol. 15, no. 23, pp. 7330–7334, 2009.

[57] N. Anfossi, P. André, S. Guia et al., "Human NK cell education by inhibitory receptors for MHC class I," *Immunity*, vol. 25, no. 2, pp. 331–342, 2006.

[58] R. Kiessling, E. Klein, and H. Wigzell, "'Natural' killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype," *European Journal of Immunology*, vol. 5, no. 2, pp. 112–117, 1975.

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Review Article

Natural Killer Cells in Human Cancer: From Biological Functions to Clinical Applications

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Natural killer (NK) cells are central components of the innate immunity. In murine models, it has been shown that NK cells can control both local tumor growth and metastasis due to their ability to exert direct cellular cytotoxicity without prior sensitization and to secrete immunostimulatory cytokines like IFN-γ. The latter participates in cancer elimination by inhibiting cellular proliferation and angiogenesis, promoting apoptosis, and stimulating the adaptive immune system, and it is instrumental for enhancing Ag processing and presentation. Nevertheless, NK cells display impaired functionality and capability to infiltrate tumors in cancer patients. Also, NK cells are feasible targets of stimulation to participate in immunotherapeutic approaches like antibody-based strategies and adoptive cell transfer. Thus, multiple attempts currently aim to manipulate NK for utilization in the immunotherapy of cancer.

1. Introduction

NK cells are effector lymphocytes of the innate immune system that control several types of tumors by limiting their growth and dissemination [1]. *In vitro* studies using cells from humans and several other mammalian species, as well as *in vivo* studies in mice and rats, have long provided evidence that tumor cells are recognized as NK cell targets [2]. There are numerous studies in mice supporting the notion that NK cells are involved in the elimination of tumor cells. The *in vivo* analyses relied on antibody-mediated depletion of NK cells in mice, targeting either NK1.1 or the glycolipid asialo-GM1 [3–5]. However, NK cell depletion with antibodies to NK1.1 may also affect populations of invariant natural killer T cells and other NK1.1$^+$ T-cell populations. The selectivity of NK cell depletion with asialo-GM1 antibodies (Abs) has also been hampered by the expression of asialo-GM1 by several cell types including myeloid cells, epithelial cells, and T-cell subsets. Caution is therefore required when interpreting studies based upon Ab depletion because of the lack of specificity of Ab treatment against NK populations. Nevertheless, many other independent studies advocate a role for NK cells in the control of tumor development in mice. Particularly, mouse NK cells are involved in the *in vivo* rejection of several transplanted tumors, in a manner dependent upon the presence or absence of NK cell receptor ligands expressed by the tumor [6]. More informative studies regarding tumor immunosurveillance are the experiments addressing the control of newly arising tumors. In this respect Schreiber's group demonstrated that frequencies of spontaneously arising tumors or induced by methylcholantrene (MCA) were higher in mice deficient for key effector molecules of NK cells or the respective receptors [7, 8]. Nevertheless, in a further study from the same group, it was shown that although NK cells are important in the early elimination of MCA-induced tumors, control of the "dormant" tumor state depends mainly on adaptive immunity [9]. Other studies provided evidence that NK cells can recognize and eliminate aberrant cells. The lack of MHC class I expression [10] or the upregulation of NKG2D ligands [11, 12] can render tumor cells susceptible to NK cell-mediated lysis. In some of

these experimental models, NK cell-mediated elimination of tumor cells induced the subsequent development of tumor-specific T-cell responses to the parental tumor cells [11, 13] as a bridge between innate and adaptive immune responses [14]. A role for NK cells in tumor immunosurveillance has also been implicated in controlling the growth of B-cell lymphomas that spontaneously arise in mice lacking both perforin and β2-microglobulin [15]. Moreover, the blocking of NK cell MHC class-I-specific inhibitory receptors increases NK cell effector function against tumor cells in mice [16]. In addition to their endogenous protective role in tumor models, NK cells are also mediators of the antitumor effects of several recombinant cytokines, such as IL-2, IL-12, IL-18, and IL-21 [6]. So far, these studies *in vitro* and in animal models show the role of NK cells in cancer immunosurveillance.

Clinical and experimental evidence demonstrate that this important role of NK cells holds true in humans. Although the paucity of NK cell-selective deficiencies has limited the characterization of NK cell biological function *in vivo* in general and in antitumor immunosurveillance in particular [17], there is an 11-year follow-up epidemiologic survey which has shown that the extent of NK cell activity in peripheral blood is associated with cancer risk in adults: low NK cell activity is associated with increased cancer risk [18]. Furthermore, intratumoral NK cell studies have been hampered because of the low numbers of these cells and the difficulty to obtain them from tumor samples. However, in the last few years, novel studies have revealed the phenotypic status and functionality of NK cells in tumor site and also in peripheral blood (PB) of cancer patients. First studies about tumor immune infiltration have shown that the presence of NK cells represents a positive prognostic marker in different carcinomas [19–21]. Further studies in established human tumors showed that there are often only a few infiltrating NK cells which are unlikely to greatly contribute to the elimination of tumor cells [22, 23]. It has been suggested that despite of low NK cell numbers in tumors, due to their inefficient homing into malignant tissues, this situation may be overcome by cytokine-mediated activation in immunotherapeutical regimens [24]. In this respect, all this evidence about NK importance in tumor immune control enhances the appeal of NK cell-based immunotherapeutic approaches. For instance, the beneficial role of NK cells in controlling human malignancies stems from clinical studies of leukemia patients who received alloreactive NK cells in the course of allogeneic hematopoietic stem cell transplantation [25, 26]. Likewise, several studies have established the importance of Fc-FcγR interactions for the *in vivo* antitumor effects of certain monoclonal antibodies [27–30]. In the present work we will revise key aspects of NK cells role from molecular and cellular characteristics to therapeutic applications in cancer patients.

2. NK Cells in Cancer Patients

2.1. NK Cells Tumor Infiltration. Primary tumor growth is a complex process, involving many interactions between the tumors and surrounding tissue. A developing tumor influences and is influenced by its stroma, initiates angiogenesis, and interacts with both the adaptive and innate immune systems. The clinicopathological significance of the tumor-infiltrating lymphocytes (TILs) in various human cancers has been an issue of great interest. $CD8^+$ cytotoxic T cells (CTL) and NK cells are the most likely effectors for an effective antitumor immunity [31]. Several studies have shown that the infiltration of lymphocytes significantly correlates with a prolonged survival time of patients, at least in certain types of cancer [32–34]. Typically, NK cells are not found in large numbers in advanced human neoplasms, indicating that they do not normally home efficiently to malignant tissues. For instance, a low prevalence of gastric and colorectal (CRC) tumor-infiltrating $CD56^+$ cells in livers with multiple metastases was detected, whereas in cases with solitary metastases a higher degree of lymphocyte infiltration was observed. Moreover, the percentage of intrahepatic NK ($CD56^+$) cells was also decreased in patients with metastases compared to those without, being almost twice lower than $CD8^+$ and $CD4^+$. This suggests that low NK cell number could be a reason for the escape of metastatic cells from the mechanisms of liver immune control [35]. In human non-small cell lung cancers (NSCLC) NK cells percentage within $CD45^+$ mononuclear cells (MCs) isolated from lung tumors was consistently lower than in MC from the PB counterpart and was comparable to that in MC from peritumoral lung tissue. With regard to their localization, NK cells were found in tumor stroma, whereas they were not in direct contact with cancer cells [36]. Another study in NSCLC showed that NK cell infiltration was strikingly heterogenous. Malignant and nonmalignant tissue areas in NSCLC were selectively infiltrated by certain immune cell types with NK cells being displaced from the tumor tissue and displaying low cytotoxic activity [23]. In another study, the $CD56^+/CD16^+$ cell ratio in renal cell carcinoma microenvironment was found generally lower than 1, suggesting that a predominant number of $CD16^+$ cells were $CD56^-$ macrophages, and a low NK cell infiltration [37].

2.2. Tumor-Associated NK Cell Phenotype. The limited number of NK cells infiltrating tumors has hampered more extensive *ex vivo* analyses of such tumor-associated cells. However, novel studies of tumor-associated NK cells demonstrated a striking phenotype, supporting the notion that tumor-induced alterations of activating NK cell receptor expression may hamper immune surveillance and promote tumor progression. In this sense, several authors studied the balance between inhibitory and activating receptors of NK cells that infiltrate tumors, observing in some cases downregulation of activating receptors and in others, overexpression of inhibitory ones. In a study which analyzed the receptor repertoire and functional integrity of NK cells in peritoneal effusions from patients with ovarian carcinoma, tumor-associated NK cells expressed reduced levels of the activating DNAM-1, 2B4, and CD16 receptors and were hyporesponsive to HLA class I-deficient K562 cells and to coactivation via DNAM-1 and 2B4, relative to autologous peripheral blood NK cells. Moreover, tumor-associated NK cells were

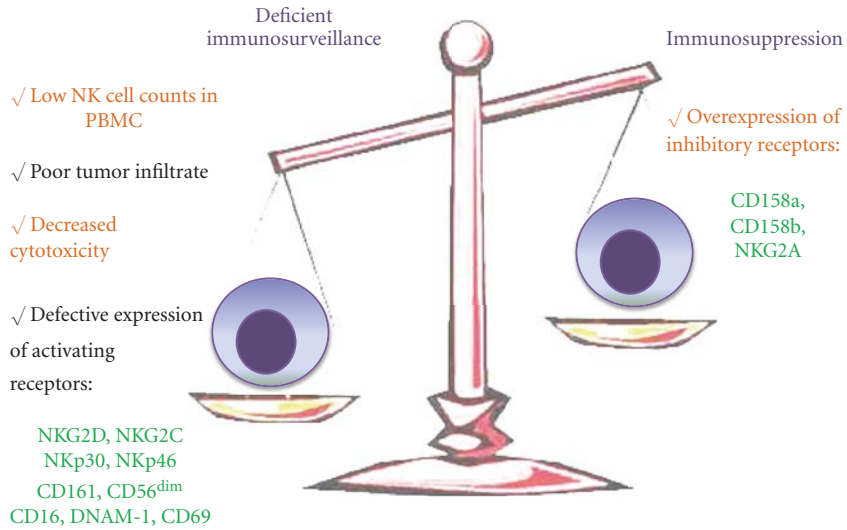

Figure 1: NK cell abnormalities in cancer patients. A defective immunity secondary to tumor development has been well established in different types of cancer. The imbalance of immune status is inclined to immunosuppression in cancer patients which results in a concomitant tendency to tumor immune evasion. Such immunosuppression is characterized by a decrease in NK cell numbers in PB and a decreased in tumor infiltrate as compared to normal tissue. Moreover, in many types of cancer an altered phenotype which presents defective expression of activating receptors and overexpression of inhibitory receptors is observed.

also refractory to CD16 receptor stimulation, resulting in diminished Ab-dependent cellular cytotoxicity (ADCC) against autologous tumor cells [38]. In another study, tongue cancer intraepithelial NK cells expressed NKG2A, an inhibitory receptor that recognizes HLA-E, more frequently than those in the stroma or in lichen planus. Collectively, the intraepithelial $CD8^+$ T cells and NK cells were phenotypically inactivated, whereas stromal counterparts were phenotypically just as active as those in lichen planus, suggesting the suppressed state of the intraepithelial NK cells [39]. Carrega and coworkers described both relevant molecule expression and function of NK cells infiltrating NSCLC in comparison with autologous NK cells isolated from either peritumoral normal lung tissues or PB. The $CD56^{bright}CD16^-$ NK cell subset was highly enriched in tumor infiltrate and displayed activation markers, including NKp44, CD69, and HLA-DR. Remarkably, the cytolytic potential of NK cells isolated from cancer tissues was lower than that of NK cells from PB or normal lung tissue, whereas no difference was observed regarding their capability of producing cytokines [36]. In acute myeloid leukemia (AML) it was demonstrated that CD137 ligand (CD137L) was expressed on leukemic cells and interacts with CD137 on activated NK cells. Bidirectional signaling following CD137-CD137L interaction induced the release of the immunomodulatory cytokines IL-10 and TNF by AML cells and directly diminished granule mobilization, cytotoxicity, and IFN-γ production by human NK cells, demonstrating an immune evasion of AML cells by impairing NK-cell tumor surveillance [40].

2.3. Peripheral Blood NK Phenotype. Not only did NK infiltrating tumor cells show a different phenotype or reduced cytotoxicity, but in some kind of cancers this was also observed in NK cells from PB. In a study in metastatic melanoma (MM) patients, NK cells presented decreased activity and IFN-γ production and also had a redistribution of NK cell subsets. It was observed an increase in non-cytotoxic $CD16^{dim}CD56^{bright}$ and a reduction in cytotoxic $CD16^{bright}CD56^{dim}$ NK cell subsets. Moreover, there was a decreased CD161 and NKG2D activating receptors and an overexpressed CD158a inhibitory NK cell receptor, which correlated to lower NK cell cytotoxicity [41]. These results are in agreement with another study in MM where a significantly lower percentage of NK cells expressed CD16, NKp30, and NKp46 activating receptors as compared to healthy donors. In addition, despite the importance of NKG2D in recognizing MM cells, no substantial differences were observed between stage IV MM patients and healthy donors [41]. These observations imply that down-regulated NK litic receptor expression in MM patients may affect NK cells ability to recognize and eliminate tumor cells [42]. Likewise, the frequency of NK cells expressing the activating receptors NKp30, NKp44, NKp46, NKG2D, and NKG2C was significantly decreased in AML patients compared to the NK cells of normal controls [43]. Although the molecular mechanisms responsible for the reduced receptor expression in PB NK cells remains elusive, elevated serum levels of soluble NK cell receptor ligands shed by tumor cells have been associated with downregulation of the NK cell receptors and might contribute to the decreased levels of NK cell activity [44].

In this section, we cited works which showed some impaired NK cells characteristics in cancer patients which determine their inability to eliminate tumor cells (depicted in Figure 1). On the other hand, it is a fact that NK

cells are potentially active against tumor cells and it is feasible to manipulate them to restore and/or improve their antitumoral activity. NK cell-based immunotherapeutic approaches will be reviewed in next section.

3. NK Cell Therapy

3.1. Monoclonal Antibodies. Many mechanisms have been proposed to explain the clinical antitumor activity of unconjugated tumor antigen-specific monoclonal Abs (MAbs). The ability of some MAbs to disrupt signalling pathways involved in the maintenance of the malignant phenotype has received widespread attention. In addition, Abs exhibit various immunomodulatory properties and, by directly activating or inhibiting molecules of the immune system, they can promote the induction of antitumor immune responses. However, this ability has been less studied. In this section, we will describe NK cell-mediated ADCC mechanism and discuss the potential use of MAbs to manipulate the host immune response to tumors.

3.2. Antibody-Dependent Cellular Cytotoxicity. Abs are linked to immune effector functions by the Fc fragment, which is capable of initiating ADCC when binding to Fc receptors, specially FcγRIII (CD16) on NK cells, which initiates a sequence of cellular events culminating in the release of cytotoxic granzyme-containing granules and INF-γ secretion [45–47]. Several studies have established the importance of Fc-FcγR interactions for the antitumor effects of certain MAbs in murine models and clinical trials. A seminal paper showed that the antitumor activities of the anti-HER-2 MAb, Trastuzumab, and the anti-CD20 MAb, Rituximab, were lower in FcγR-deficient mice than in wild-type mice [48]. The role of FcγR in the antitumor response has been further supported by the finding that polymorphisms in the gene encoding FcγRIII, which lead to higher binding of Ab to FcγRIII, are associated with high response rates to Rituximab in patients with follicular non-Hodgkin's lymphoma [27]. A separate study that compared clinical responses to Rituximab in patients with follicular lymphoma suggested that both FcγRIII and FcγRIIB have a role in the response to Rituximab [28]. More recent findings show that polymorphisms in genes encoding FcγRs are associated with clinical responses to other Abs, including Trastuzumab [29] and the anti-EGFR MAb, Cetuximab [30]. Patients with breast cancer who responded to Trastuzumab with complete or partial remission have been found to have a higher capability to mediate *in vitro* ADCC in response to Trastuzumab than patients whose tumors failed to respond to therapy [29]. ADCC enhancement through Fc domain modification has shown promise in the development of next generation MAbs. For example, a CD19-specific MAb with increased FcγRIIIA binding affinity mediated significantly increased ADCC compared to its parental MAb and Rituximab [49]. The *in vivo* infusion of this high affinity MAb efficiently cleared malignant B cells in cynomolgus macaques (*Macaca fascicularis*) [50].

3.3. Potentiating ADCC. In previous reports, we and others proved that NK cells produce Cetuximab-mediated ADCC of metastatic CRC (mCRC) and that this activity is not affected by the mutational status of the downstream molecule K-RAS [51, 52]. This effect is expected because NK cells recognize the surface-bound Abs and are able to kill tumor cells independently of the EGFR pathway activation. Nevertheless, it remains to be answered why in mCRC patients with K-RAS mutated status Cetuximab-mediated ADCC does not induce clinical remissions. As we discussed, one of the possibilities is the low proportion of NK cells infiltrating CRC tumors [53] and the low functional capacity of these cells observed in cancer patients [54, 55]. Therefore, if it were possible to enhance the activity of NK cells, the efficacy of treatment with Cetuximab could be increased. The anti-VEGF-A MAb, Bevacizumab, is also active in mCRC, regardless of the K-RAS mutation status; nevertheless, it needs to be administered in combination with a cytotoxic agent [56]. Regarding ADCC enhancer molecules, Lenalidomide, an analog of thalidomide, is able to potentiate ADCC *in vitro*. Lenalidomide enhanced NK cell and monocyte-mediated ADCC of Rituximab against a variety of hematological cell lines *in vitro* [57]. Lenalidomide also enhanced NK cell-mediated lysis of Cetuximab and Trastuzumab-coated CRC and breast cancer cells, respectively. The ability of lenalidomide to enhance Cetuximab-mediated ADCC of CRC cells was not affected by the K-RAS mutational status [58]. Because panitumumab, an IgG2a anti-EGFR MAb, does not effectively interact with Fcγ receptors on the NK cell surface, it was unable to initiate ADCC and, as expected, lenalidomide had no effect because its activity is reliant on the augmentation of NK cell signaling downstream of FcγR. An early clinical study exploring this effect of lenalidomide in K-RAS mutant tumors has been initiated [59].

As explained previously, many studies demonstrated the impairment of NK cell activity in cancer patients. In these cases, cytokines could restore and potentiate NK cell-mediated ADCC. The works cited below explain how these treatments work when patients present NK cell dysfunction. Cetuximab was tested in various *in vitro* studies in patients with esophageal squamous cell carcinoma (ESCC) to evaluate the possibility of treatment. Authors first performed detailed analysis of ADCC mediated by Cetuximab against ESCC cell lines with various levels of EGFR. The activities of Cetuximab-mediated ADCC by patients' PBMC were impaired in comparison with those by healthy donors' PBMC. Moreover, the inhibition of transforming growth factor (TGF)-β could enhance Cetuximab-mediated ADCC against TGF-β-producing ESCC [60]. In other work, downregulated CD16 and upregulated CD56 molecules on NK cells were observed in ESCC patients, resulting in NK cell dysfunction. After patients received curative resections of ESCC, the downregulated CD16 and upregulated CD56 were significantly restored to the levels of healthy donors. TGF-β1 was found to partially contribute to downregulation of CD16 on NK cells [61]. Watanabe and coworkers evaluated whether IL-21 could improve the impairment of ADCC in patients with ESCC as IL-21 was reported to have the

ability to activate NK cells. Trastuzumab- and Cetuximab-mediated ADCC of PBMC or of enriched NK cells was enhanced by the addition of IL-21 in a dose-dependent manner and the levels of ADCC enhanced by IL-21 in patients were high enough in comparison with those in healthy donors, paralleling the upregulation of CD247 on NK cells [55]. As in the previous example, NK cell response to Cetuximab-coated tumor cells could be enhanced by the administration of NK cell stimulatory cytokines IL-2, IL-12, or IL-21 and resulted in higher IFN-γ production than was observed with either agent alone. NK cell-derived IFN-γ significantly enhanced monocyte ADCC against Cetuximab-coated tumor cells. Costimulated NK cells also secreted elevated levels of chemokines (IL-8, macrophage inflammatory protein-1a, and RANTES) that could direct the migration of naive and activated T cells. Furthermore, administration of IL-21 enhanced the effects of Cetuximab in a murine tumor model [62]. In other experimental study, it was analyzed the correlation between EGFR expression in lung cancer cell lines and the ADCC activity of Cetuximab as well as the influence of IL-2 and chemotherapy on ADCC activity. A logarithmic correlation was observed between the number of EGFRs and ADCC activity. In addition, NK cell-mediated ADCC was enhanced by IL-2 and such cells were also less susceptible to immunosuppression by chemotherapy than in lung cancer patients [63]. The antitumor effect and mechanism of action of Cetuximab using EGFR high-expressing and EGFR low-expressing gastric cancer cell lines without gene amplification was investigated. Cetuximab showed neither significant growth inhibition nor induction of apoptosis in either cell line *in vitro*, and only slightly inhibited ligand-induced phosphorylation of protein kinase B and extracellular signal-regulated kinase. In contrast, Cetuximab significantly inhibited subcutaneous and intraperitoneal tumor growth in *nude* mice. This antitumor activity was significantly enhanced and diminished by treatment with IL-2 and antiasialo GM1 Ab, respectively [64]. In that study, HER-2/*neu*-expressing gastric cancer cells could be killed by Herceptin, the anti-HER-2/*neu* MAb. Herceptin-mediated ADCC correlated with the degree of HER-2/*neu* expression on the gastric cancer cells. However, the Herceptin-mediated ADCC was significantly impaired in PBMC from advanced disease patients compared with that in early disease or healthy individuals. Moreover, NK cells purified from patients with advanced disease indicated less Herceptin-mediated ADCC in comparison with that from healthy donors, whereas monocytes purified from the patients showed an almost equal amount of Herceptin-mediated ADCC in comparison with that from healthy individuals, indicating that NK cell dysfunction contributed to the impaired Herceptin-mediated ADCC in gastric cancer patients. Furthermore, the NK-cell dysfunction on Herceptin-mediated ADCC correlated with the downregulation of CD16 expression in the patients, and IL-2 *ex vivo* treatment of NK cells could restore the impairment of Herceptin-mediated ADCC, concomitant to the normalization of the expression of CD16 molecules [65]. In our laboratory, we performed *in vitro* studies on human triple negative breast cancer (TNBC) K-RAS-mutated cell lines. We found that EGFR-expressing TNBC could be killed by Cetuximab-mediated ADCC at clinically achievable concentrations. Furthermore, IL-15 could replace IL-2 in most of its immunologic activities, stimulating NK cells ability to produce IFN-γ, paralleling the upregulation of activating receptors [66]. These results show that Cetuximab-mediated NK cell activity can be significantly enhanced in the presence of NK cell stimulatory cytokines.

3.4. Adoptive Cell Transfer. Adoptive cell transfer (ACT) therapy can be considered as a strategy aimed at replacing, repairing, or enhancing the biological function of the immune system by means of autologous or allogeneic cells. ACT therapy may include (i) removal or enrichment of various cell populations; (ii) expansion of hematopoietic cell subsets; (iii) activation of lymphocytes for immunotherapy; (iv) genetic modification of lymphoid cells, when these cells are intended to engraft transiently in the recipient and/or be used in the treatment of cancer. This section contains extensive considerations on clinical and laboratory experience in immunologic targeting of malignant cell populations, and how lasting curative responses can be effectively generated. As we will discuss, the use of lymphocytes and/or NK cells as a strategic weapon in preventing or curing the neoplastic relapse after surgery/chemotherapy has been applied to the treatment of hematological malignancies and solid tumors.

NK cells represent a promising cell type to utilize for effective adoptive immunotherapy. Transfer of tumor-infiltrating lymphocytes has shown some remarkable responses in patients with advanced MM [67]. Nevertheless, problems with deriving sufficient numbers of these cells from patients, downregulation of MHC class I ligands and costimulatory ligands on tumor cells, combined with the lack of persistence of transferred cells have precluded broad utilization of these cells for the treatment of cancer patients. The transfer of other immune subsets, such as NK cells which do not require prior sensitization to respond to target cells and can potently induce a cytolytic response, represents a good alternate or auxiliary cell type for immunotherapy.

3.5. Ex Vivo Expansion. One of the main hurdles involved in developing adoptive transfer of immune cells has been to define good manufacturing procedures (GMPs) compliant methods to isolate defined subsets with the number of cells required that guarantee the safety of the injection to patients. In this respect, clinical-grade production of NK cells has proven efficient [68], and large-scale expansion method has been possible for human NK cells [69], using a cytokine-based culture system for *ex vivo* expansion of NK cells from hematopoietic progenitor cells from umbilical cord blood. Systematic refinement of this two-step system using a novel clinical-grade medium resulted in a therapeutically applicable cell culture protocol. Nevertheless, improved technologies for NK cells expansion lately have been tested. NK cells from myeloma patients expanded in a bioreactor displayed significantly higher cytotoxic capacity. It was possible to partially attribute this to a higher expression level of NKp44 compared with NK cells expanded in flasks [70]. These results demonstrate that large amounts of highly active

NK cells for adoptive immunotherapy can be produced in a closed, automated, large-scale bioreactor under feeder-free current GMP conditions, facilitating clinical trials for the use of these cells.

Even when the first works were based on transplantation of autologous *ex vivo* expanded cells, and in addition to what we earlier discussed on cancer patients NK cells dysfunctions, it is now understood that the failure of autologous NK therapy is partially due to the downregulation of NK cell killing that occurs with recognition of self-class I MHC on tumor cells [71–73], making allogeneic cell transfer more attractive. During allogeneic hematopoietic stem cell transplantation, NK cells have been implicated in the suppression of Graft versus Host Disease (GVHD), the promotion of bone marrow engraftment, and mediation of a Graft versus Leukemia (GVL) effect [74]. In the setting of allogeneic ACT in leukemias, the results of NK cells activity depend on the directionality of lysis [75, 76]. When the NK cells are donor-derived and the recipient cells lack expression of the cognate KIR ligand, the donor NK cell lysis of recipient target cells can result in GVL and/or GVHD, depending on the tissue origin of the NK cell target. However, if the target cell is of donor origin, and the NK effector cell is of recipient origin, NK cell lysis can result in graft rejection. Recent studies have demonstrated that NK cell effector capacity is influenced by class and quantity of inhibitory receptors for self-HLA-B and HLA-C ligands [77, 78]. It has been estimated that NK cell alloreactivity can be expected to occur in about 50% of unrelated donor transplants with one or more HLA allele level mismatches. Velardi and coworkers announced a new era in the exploitation of NK cells for cancer immunotherapy with a pioneering study of hematopoietic stem cell transplantation that stratified patients according to KIR-ligand mismatch between donor cells and recipient leukemic cells [74]. This reveals that alloreactive NK cells, unrestrained by inhibitory signals from the recipient HLA ligands, protects against disease relapse. This KIR-ligand mismatch phenomenon has attracted researchers to study it, and similar observations have since been made in other trials of leukemia immunotherapy [79–81]. On the other hand, further studies extended HLA match analysis to the mechanisms involved in GVHD. In patients with hematologic malignancies who received transplants from unrelated donors, genotype analysis of six major HLA loci identified 4 HLA-C and 6 HLADPB1 mismatch combinations responsible for a decreased risk of relapse and severe acute GVHD. Donor selection made in consideration of these results might allow the separation of GVL from acute GVHD, especially in HLA-DPB1 mismatch combinations [82]. These findings might be crucial to elucidating the mechanism of the decreased risk of relapse on the basis of HLA molecule haplotype (Figure 2).

The effectiveness of adoptive transfer of expanded NK cells for the treatment of relapsed leukemia has been demonstrated, and ongoing efforts are designed to evaluate this approach in the treatment of solid tumor malignancies as well [83–85]. Despite this therapy has been proven safe for patients and feasible, more effective strategies to augment *in vivo* NK cell persistence and expansion are needed to test the clinical benefit of NK cells against solid tumors, as literature supports the notion that there is a cell dose response in ACT. In a phase II clinical trial in patients with ovarian and breast cancer, the adoptive transfer of haploidentical NK cells after lymphodepleting chemotherapy was associated with transient donor chimerism that was not improved with the addition of low-dose total body irradiation. Sustained *in vivo* NK cell expansion may be limited by host rejection, competition with host lymphocytes or suppression by recipient regulatory T cells (Treg) or myeloderived suppressor cells [86]. To provide a greater number of NK cells with a differentiated activated phenotype for adoptive immunotherapy, some authors described cytokine-based methods useful for clinical-scale NK cell production. A phase I clinical study evaluated the safety of donor lymphocyte infusions following allogeneic hematopoietic stem cell transplantation to patients with progressive malignant disease. This study reported the safety of *ex vivo*-expanded NK and NK-like T cells with a feeder-free cGMP compatible expansion strategy administered to humans also in combination with IL-2 [87]. Decot and coworkers described an immunomagnetic technique consisting in CD3/CD19 depletion effective to obtain highly T- and B-cell-depleted NK cell-enriched product with GMP-compliant reagents. IL-2 or IL-15 were equivalent to significantly enhance NK cell cytotoxicity after culture with MHC-class I negative erythroleukemia cell line. Furthermore, they observed a modification in NK-cell receptor expression pattern with upregulation of the activating receptor NKG2D, but also of the inhibiting receptors KIR2DL1 and KIR2DL2 [88]. In other work PBMC were cultured in serum-free medium and IL-2 for 20 days. Cells in the culture were also stimulated with anti-CD3 Ab (OKT3). Safety and feasibility of administering *ex vivo* expanded NK and NK-like T-effector cells as donor lymphocytes infusion to five cancer patients after stem cell transplantation was evaluated. Cell infusions, with or without IL-2 injections, seemed to be safe as no GVHD was observed. Not only do all these data support a role of NK cell during ACT therapy, but results also link NK cell recovery with a GVL rather than with a GVHD effect, as recipient's NK cells have been shown to be the first of lymphoid lineage cells to reconstitute following allogeneic transplantation, and adequate recovery of NK cell number in the early posttransplant period has been associated with improved relapse-free outcome [89].

These studies now provide the backdrop for the development of therapies that enhance the therapeutic benefit of allogeneic transplantation while minimizing the risks and toxicities associated with treatment (Figure 3).

3.6. Concluding Remarks. In recent years there have been significant advances in the discovery of the molecular mechanisms that govern the reactivity of NK cells against tumors. In this paper we have reviewed how the expression and function of both inhibitory and activating NK receptors are involved in the molecular regulation of innate immunity in malignant settings.

NK cells are also potential tools for cancer therapy, both due to cytotoxic ability enhanced by antibodies and

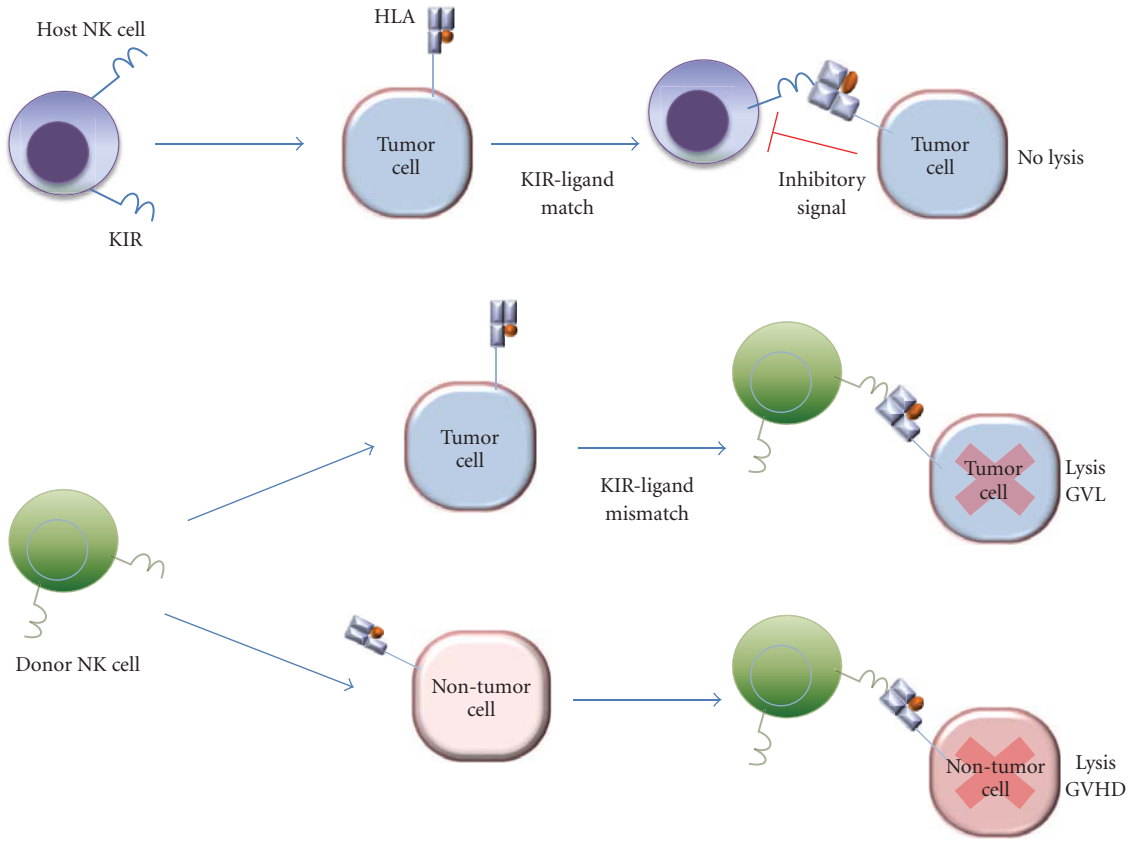

Figure 2: NK cell tumor immunity and alloimmunity after allogeneic ACT. Autologous NK cell killing is inhibited when self-class I MHC on tumor cells is recognized. In allogeneic ACT, NK cell KIR-mismatch is implicated both in GVHD and in mediating of a GVL effect.

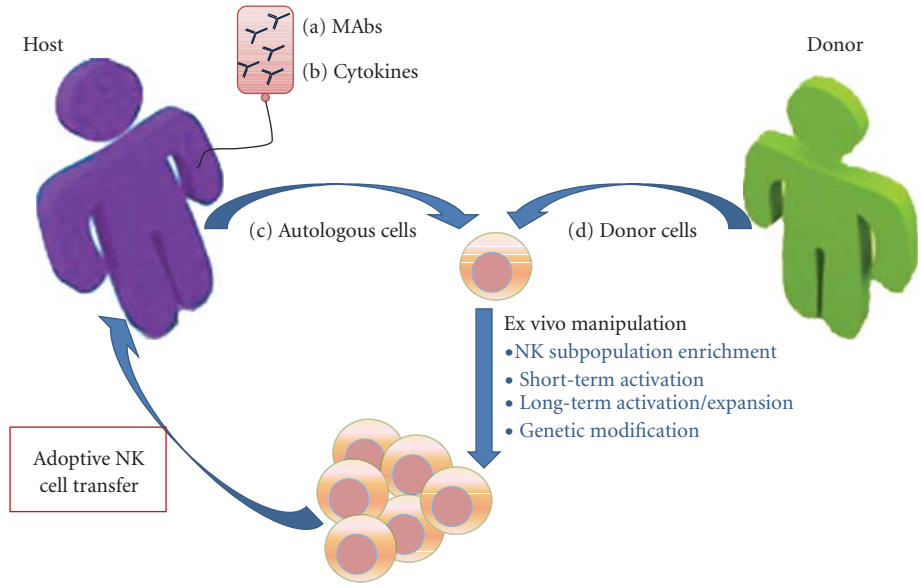

Figure 3: NK cell-based immunotherapies. Overview of current approaches being tested in clinical settings. (a) Infusion of tumor-specific MAbs triggers an ADCC response. (b) ADCC can be potentiated with the coadministration of cytokines. (c) Autologous and (d) allogeneic transfer of NK cells, which can be manipulated *ex vivo* with different approaches.

the possibility of *ex vivo* expansion and adoptive transfer. Nevertheless therapeutical regimens need to be further developed. Inasmuch as one scheme will not be suitable for all malignancies, different approaches are to be evaluated for each particular case.

Conflicts of Interests

authors declared that there are no conflict of interests.

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References

[1] E. Vivier, E. Tomasello, M. Baratin, T. Walzer, and S. Ugolini, "Functions of natural killer cells," *Nature Immunology*, vol. 9, no. 5, pp. 503–510, 2008.

[2] G. Trinchieri, "Biology of natural killer cells," *Advances in Immunology*, vol. 47, pp. 187–376, 1989.

[3] S. Kim, K. Iizuka, H. L. Aguila, I. L. Weissman, and W. M. Yokoyama, "In vivo natural killer cell activities revealed by natural killer cell-deficient mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 6, pp. 2731–2736, 2000.

[4] M. J. Smyth, Y. Hayakawa, K. Takeda, and H. Yagita, "New aspects of natural-killer-cell surveillance and therapy of cancer," *Nature Reviews Cancer*, vol. 2, no. 11, pp. 850–861, 2002.

[5] J. Wu and L. L. Lanier, "Natural killer cells and cancer," *Advances in Cancer Research*, vol. 90, pp. 127–156, 2003.

[6] C. A. Stewart and E. Vivier, "Strategies of NK cell recognition and their roles in tumor immunosurveillance," in *How the Immune System Recognizes Self and Nonself: Immunoreceptors and Their Signaling*, D. Kitamura, Ed., pp. 37–81, Springer, Tokyo, Japan, 2007.

[7] D. H. Kaplan, V. Shankaran, A. S. Dighe et al., "Demonstration of an interferon γ-dependent tumor surveillance system in immunocompetent mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 13, pp. 7556–7561, 1998.

[8] V. Shankaran, H. Ikeda, A. T. Bruce et al., "IFNγ, and lymphocytes prevent primary tumour development and shape tumour immunogenicity," *Nature*, vol. 410, no. 6832, pp. 1107–1111, 2001.

[9] C. M. Koebel, W. Vermi, J. B. Swann et al., "Adaptive immunity maintains occult cancer in an equilibrium state," *Nature*, vol. 450, no. 7171, pp. 903–907, 2007.

[10] K. Karre, H. G. Ljunggren, G. Piontek, and R. Kiessling, "Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy," *Nature*, vol. 319, no. 6055, pp. 675–678, 1986.

[11] A. Diefenbach, E. R. Jensen, A. M. Jamieson, and D. H. Raulet, "Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity," *Nature*, vol. 413, no. 6852, pp. 165–171, 2001.

[12] A. Cerwenka, J. L. Baron, and L. L. Lanier, "Ectopic expression of retinoic acid early inducible-1 gene (RAE-1) permits natural killer cell-mediated rejection of a MHC class I-bearing tumor *in vivo*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 20, pp. 11521–11526, 2001.

[13] J. M. Kelly, P. K. Darcy, J. L. Markby et al., "Induction of tumor-specific T cell memory by NK cell-mediated tumor rejection," *Nature Immunology*, vol. 3, no. 1, pp. 83–90, 2002.

[14] J. C. Sun and L. L. Lanier, "Natural killer cells remember: an evolutionary bridge between innate and adaptive immunity?" *European Journal of Immunology*, vol. 39, no. 8, pp. 2059–2064, 2009.

[15] S. E. A. Street, Y. Hayakawa, Y. Zhan et al., "Innate Immune Surveillance of Spontaneous B Cell Lymphomas by Natural Killer Cells and $\gamma\delta$ T Cells," *Journal of Experimental Medicine*, vol. 199, no. 6, pp. 879–884, 2004.

[16] C. Y. Koh, B. R. Blazar, T. George et al., "Augmentation of antitumor effects by NK cell inhibitory receptor blockade in vitro and in vivo," *Blood*, vol. 97, no. 10, pp. 3132–3137, 2001.

[17] J. S. Orange, "Human natural killer cell deficiencies," *Current Opinion in Allergy and Clinical Immunology*, vol. 6, no. 6, pp. 399–409, 2006.

[18] K. Imai, S. Matsuyama, S. Miyake, K. Suga, and K. Nakachi, "Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population," *The Lancet*, vol. 356, no. 9244, pp. 1795–1799, 2000.

[19] S. Coca, J. Perez-Piqueras, D. Martinez et al., "The prognostic significance of intratumoral natural killer cells in patients with colerectal carcinoma," *Cancer*, vol. 79, no. 12, pp. 2320–2328, 1997.

[20] S. Ishigami, S. Natsugoe, K. Tokuda et al., "Prognostic value of intratumoral natural killer cells in gastric carcinoma," *Cancer*, vol. 88, no. 3, pp. 577–583, 2000.

[21] F. R. Villegas, S. Coca, V. G. Villarrubia et al., "Prognostic significance of tumor infiltrating natural killer cells subset CD57 in patients with squamous cell lung cancer," *Lung Cancer*, vol. 35, no. 1, pp. 23–28, 2002.

[22] P. A. Albertsson, P. H. Basse, M. Hokland et al., "NK cells and the tumour microenvironment: implications for NK-cell function and anti-tumour activity," *Trends in Immunology*, vol. 24, no. 11, pp. 603–609, 2003.

[23] G. Esendagli, K. Bruderek, T. Goldmann et al., "Malignant and non-malignant lung tissue areas are differentially populated by natural killer cells and regulatory T cells in non-small cell lung cancer," *Lung Cancer*, vol. 59, no. 1, pp. 32–40, 2008.

[24] M. Hokland, J. Kjaergaard, P. J. K. Kuppen et al., "Endogenous and adoptively transferred A-NK and T-LAK cells continuously accumulate within murine metastases up to 48 h after inoculation," *In Vivo*, vol. 13, no. 3, pp. 199–204, 1999.

[25] K. C. Hsu, C. A. Keever-Taylor, A. Wilton et al., "Improved outcome in HLA-identical sibling hematopoietic stem-cell transplantation for acute myelogenous leukemia predicted by KIR and HLA genotypes," *Blood*, vol. 105, no. 12, pp. 4878–4884, 2005.

[26] L. Ruggeri, A. Mancusi, E. Burchielli, F. Aversa, M. F. Martelli, and A. Velardi, "Natural killer cell alloreactivity in allogeneic hematopoietic transplantation," *Current Opinion in Oncology*, vol. 19, no. 2, pp. 142–147, 2007.

[27] G. Cartron, L. Dacheux, G. Salles et al., "Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcγrIIIa gene," *Blood*, vol. 99, no. 3, pp. 754–758, 2002.

[28] W. K. Weng and R. Levy, "Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma," *Journal of Clinical Oncology*, vol. 21, no. 21, pp. 3940–3947, 2003.

[29] A. Musolino, N. Naldi, B. Bortesi et al., "Immunoglobulin g fragment c receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer," *Journal of Clinical Oncology*, vol. 26, no. 11, pp. 1789–1796, 2008.

[30] F. Bibeau, E. Lopez-Crapez, F. D. Fiore et al., "Impact of fcγRIIa-fcγRIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan," *Journal of Clinical Oncology*, vol. 27, no. 7, pp. 1122–1129, 2009.

[31] L. Zitvogel, A. Tesniere, and G. Kroemer, "Cancer despite immunosurveillance: immunoselection and immunosubversion," *Nature Reviews Immunology*, vol. 6, no. 10, pp. 715–727, 2006.

[32] Y. Naito, K. Saito, K. Shiiba et al., "CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer," *Cancer Research*, vol. 58, no. 16, pp. 3491–3494, 1998.

[33] E. Sato, S. H. Olson, J. Ahn et al., "Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 51, pp. 18538–18543, 2005.

[34] J. Galon, A. Costes, F. Sanchez-Cabo et al., "Type, density, and location of immune cells within human colorectal tumors predict clinical outcome," *Science*, vol. 313, no. 5795, pp. 1960–1964, 2006.

[35] M. Gulubova, I. Manolova, D. Kyurkchiev, A. Julianov, and I. Altunkova, "Decrease in intrahepatic CD56$^+$ lymphocytes in gastric and colorectal cancer patients with liver metastases," *Acta Pathologica, Microbiologica et Immunologica*, vol. 117, no. 12, pp. 870–879, 2009.

[36] P. Carrega, B. Morandi, R. Costa et al., "Natural killer cells infiltrating human nonsmall-cell lung cancer are enriched in CD56 bright CD16- cells and display an impaired capability to kill tumor cells," *Cancer*, vol. 112, no. 4, pp. 863–875, 2008.

[37] G. Sconocchia, G. C. Spagnoli, D. Del Principe et al., "Defective infiltration of natural killer cells in MICA/B-positive renal cell carcinoma involves β-integrin-mediated interaction," *Neoplasia*, vol. 11, no. 7, pp. 662–671, 2009.

[38] M. Carlsten, H. Norell, Y. T. Bryceson et al., "Primary human tumor cells expressing CD155 impair tumor targeting by down-regulating DNAM-1 on NK cells," *Journal of Immunology*, vol. 183, no. 8, pp. 4921–4930, 2009.

[39] F. Katou, H. Ohtani, Y. Watanabe, T. Nakayama, O. Yoshie, and K. Hashimoto, "Differing phenotypes between intraepithelial and stromal lymphocytes in early-stage tongue cancer," *Cancer Research*, vol. 67, no. 23, pp. 11195–11201, 2007.

[40] T. Baessler, J. E. Charton, B. J. Schmiedel et al., "CD137 ligand mediates opposite effects in human and mouse NK cells and impairs NK-cell reactivity against human acute myeloid leukemia cells," *Blood*, vol. 115, no. 15, pp. 3058–3069, 2010.

[41] G. Konjević, K. Mirăjaić Martinovi, V. Jurišić, N. Babović, and I. Spužić, "Biomarkers of suppressed natural killer (NK) cell function in metastatic melanoma: decreased NKG2D and increased CD158a receptors on CD3-CD16+ NK cells," *Biomarkers*, vol. 14, no. 4, pp. 258–270, 2009.

[42] G. Markel, R. Seidman, M. J. Besser et al., "Natural killer lysis receptor (NKLR)/NKLR-ligand matching as a novel approach for enhancing anti-tumor activity of allogeneic NK cells," *PLoS ONE*, vol. 4, no. 5, Article ID e5597, 2009.

[43] M. J. Szczepanski, M. Szajnik, A. Welsh, K. A. Foon, T. L. Whiteside, and M. Boyiadzis, "Interleukin-15 enhances natural killer cell cytotoxicity in patients with acute myeloid leukemia by upregulating the activating NK cell receptors," *Cancer Immunology, Immunotherapy*, vol. 59, no. 1, pp. 73–79, 2010.

[44] M. B. Fuertes, M. V. Girart, L. L. Molinero et al., "Intracellular retention of the NKG2D ligand MHC class i chain-related gene a in human melanomas confers immune privilege and prevents NK cell-mediated cytotoxicity," *Journal of Immunology*, vol. 180, no. 7, pp. 4606–4614, 2008.

[45] P. J. Leibson, "Signal transduction during natural killer cell activation: inside the mind of a killer," *Immunity*, vol. 6, no. 6, pp. 655–661, 1997.

[46] H. Terunuma, X. Deng, Z. Dewan, S. Fujimoto, and N. Yamamoto, "Potential role of NK cells in the induction of immune responses: implications for NK cell-based immunotherapy for cancers and viral infections," *International Reviews of Immunology*, vol. 27, no. 3, pp. 93–110, 2008.

[47] N. Anfossi, P. André, S. Guia et al., "Human NK cell education by inhibitory receptors for MHC class I," *Immunity*, vol. 25, no. 2, pp. 331–342, 2006.

[48] R. A. Clynes, T. L. Towers, L. G. Presta, and J. V. Ravetch, "Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets," *Nature Medicine*, vol. 6, no. 4, pp. 443–446, 2000.

[49] H. M. Horton, M. J. Bernett, E. Pong et al., "Potent *in vitro* and *in vivo* activity of an Fc-engineered anti-CD19 monoclonal antibody against lymphoma and leukemia," *Cancer Research*, vol. 68, no. 19, pp. 8049–8057, 2008.

[50] J. Zalevsky, I. W. L. Leung, S. Karki et al., "The impact of Fc engineering on an anti-CD19 antibody: increased Fcγ receptor affinity enhances B-cell clearing in nonhuman primates," *Blood*, vol. 113, no. 16, pp. 3735–3743, 2009.

[51] E. M. Levy, G. Sycz, J. M. Arriaga et al., "Cetuximab-mediated cellular cytotoxicity is inhibited by HLA-E membrane expression in colon cancer cells," *Innate Immunity*, vol. 15, no. 2, pp. 91–100, 2009.

[52] P. Correale, M. Marra, C. Remondo et al., "Cytotoxic drugs up-regulate epidermal growth factor receptor (EGFR) expression in colon cancer cells and enhance their susceptibility to EGFR-targeted antibody-dependent cell-mediated-cytotoxicity (ADCC)," *European Journal of Cancer*, vol. 46, no. 9, pp. 1703–1711, 2010.

[53] F. Pagès, A. Kirilovsky, B. Mlecnik et al., "In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer," *Journal of Clinical Oncology*, vol. 27, no. 35, pp. 5944–5951, 2009.

[54] S. Wulff, R. Pries, K. Börngen, T. Trenkle, and B. Wollenberg, "Decreased levels of circulating regulatory NK cells in patients with head and neck cancer throughout all tumor stages," *Anticancer Research*, vol. 29, no. 8, pp. 3053–3057, 2009.

[55] M. Watanabe, K. Kono, Y. Kawaguchi et al., "Interleukin-21 can efficiently restore impaired antibody-dependent cell-mediated cytotoxicity in patients with oesophageal squamous cell carcinoma," *British Journal of Cancer*, vol. 102, no. 3, pp. 520–529, 2010.

[56] H. I. Hurwitz, J. Yi, W. Ince, W. F. Novotny, and O. Rosen, "The clinical benefit of bevacizumab in metastatic colorectal cancer is independent of K-ras mutation status: analysis of a phase in study of bevacizumab with chemotherapy in previously

untreated metastatic colorectal cancer," *Oncologist*, vol. 14, no. 1, pp. 22–28, 2009.

[57] L. Wu, M. Adams, T. Carter et al., "Lenalidomide enhances natural killer cell and monocyte-mediated antibody-dependent cellular cytotoxicity of rituximab-treated CD20+ tumor cells," *Clinical Cancer Research*, vol. 14, no. 14, pp. 4650–4657, 2008.

[58] L. Wu, A. Parton, L. Lu, M. Adams, P. Schafer, and J. B. Bartlett, "Lenalidomide enhances antibody-dependent cellular cytotoxicity of solid tumor cells in vitro: influence of host immune and tumor markers," *Cancer Immunology, Immunotherapy*, vol. 60, no. 1, pp. 61–73, 2011.

[59] D. Keizman, M. Zahurak, V. Sinibaldi et al., "Lenalidomide in nonmetastatic biochemically relapsed prostate cancer: results of a phase I/II double-blinded, randomized study," *Clinical Cancer Research*, vol. 16, no. 21, pp. 5269–5276, 2010.

[60] Y. Kawaguchi, K. Kono, K. Mimura, H. Sugai, H. Akaike, and H. Fujii, "Cetuximab induce antibody-dependent cellular cytotoxicity against EGFR-expressing esophageal squamous cell carcinoma," *International Journal of Cancer*, vol. 120, no. 4, pp. 781–787, 2007.

[61] M. Watanabe, K. Kono, Y. Kawaguchi et al., "NK cell dysfunction with down-regulated CD16 and up-regulated CD56 molecules in patients with esophageal squamous cell carcinoma," *Diseases of the Esophagus*, vol. 23, no. 8, pp. 675–681, 2010.

[62] J. M. Roda, T. Joshi, J. P. Butchar et al., "The activation of natural killer cell effector functions by cetuximab-coated, epidermal growth factor receptor—positive tumor cells is enhanced by cytokines," *Clinical Cancer Research*, vol. 13, no. 21, pp. 6419–6428, 2007.

[63] J. Kurai, H. Chikumi, K. Hashimoto et al., "Antibody-dependent cellular cytotoxicity mediated by cetuximab against lung cancer cell lines," *Clinical Cancer Research*, vol. 13, no. 5, pp. 1552–1561, 2007.

[64] M. Hara, H. Nakanishi, K. Tsujimura et al., "Interleukin-2 potentiation of cetuximab antitumor activity for epidermal growth factor receptor-overexpressing gastric cancer xenografts through antibody-dependent cellular cytotoxicity," *Cancer Science*, vol. 99, no. 7, pp. 1471–1478, 2008.

[65] K. Kono, A. Takahashi, F. Ichihara, H. Sugai, H. Fujii, and Y. Matsumoto, "Impaired antibody-dependent cellular cytotoxicity mediated by Herceptin in patients with gastric cancer," *Cancer Research*, vol. 62, no. 20, pp. 5813–5817, 2002.

[66] M. P. Roberti, M. M. Barrio, A. I. Bravo et al., "IL-15 and IL-2 increase Cetuximab-mediated cellular cytotoxicity against triple negative breast cancer cell lines expressing EGFR," *Breast Cancer Research and Treatment*. In press.

[67] M. E. Dudley, J. R. Wunderlich, P. F. Robbins et al., "Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes," *Science*, vol. 298, no. 5594, pp. 850–854, 2002.

[68] D. H. McKenna, D. Sumstad, N. Bostrom et al., "Good manufacturing practices production of natural killer cells for immunotherapy: a six-year single-institution experience," *Transfusion*, vol. 47, no. 3, pp. 520–528, 2007.

[69] J. Spanholtz, M. Tordoir, D. Eissens et al., "High log-scale expansion of functional human natural killer cells from umbilical cord blood CD34-positive cells for adoptive cancer immunotherapy," *PLoS ONE*, vol. 5, no. 2, Article ID e9221, 2010.

[70] T. Sutlu, B. Stellan, M. Gilljam et al., "Clinical-grade, large-scale, feeder-free expansion of highly active human natural killer cells for adoptive immunotherapy using an automated bioreactor," *Cytotherapy*, vol. 12, no. 8, pp. 1044–1055, 2010.

[71] D. H. Raulet and W. Held, "Natural killer cell receptors: the offs and ons of NK cell recognition," *Cell*, vol. 82, no. 5, pp. 697–700, 1995.

[72] K. Karre, "Express yourself or die: peptides, MHC molecules, and NK cells," *Science*, vol. 267, no. 5200, pp. 978–979, 1995.

[73] A. Moretta, M. Vitale, C. Bottino et al., "P58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK) cells. Anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specificities," *Journal of Experimental Medicine*, vol. 178, no. 2, pp. 597–604, 1993.

[74] L. Ruggeri, M. Capanni, E. Urbani et al., "Effectiveness of donor natural killer cell aloreactivity in mismatched hematopoietic transplants," *Science*, vol. 295, no. 5562, pp. 2097–2100, 2002.

[75] K. C. Hsu and B. O. Dupont, "Natural killer cell receptors: regulating innate immune responses to hematologic malignancy," *Seminars in Hematology*, vol. 42, no. 2, pp. 91–103, 2005.

[76] L. Ruggeri, M. Capanni, A. Mancusi, M. F. Martelli, and A. Velardi, "The impact of donor natural killer cell alloreactivity on allogeneic hematopoietic transplantation," *Transplant Immunology*, vol. 14, no. 3-4, pp. 203–206, 2005.

[77] M. Pfeiffer, M. Schumm, T. Feuchtinger, K. Dietz, R. Handgretinger, and P. Lang, "Intensity of HLA class I expression and KIR-mismatch determine NK-cell mediated lysis of leukaemic blasts from children with acute lymphatic leukaemia," *British Journal of Haematology*, vol. 138, no. 1, pp. 97–100, 2007.

[78] J. Yu, G. Heller, J. Chewning, S. Kim, W. M. Yokoyama, and K. C. Hsu, "Hierarchy of the human natural killer cell response is determined by class and quantity of inhibitory receptors for self-HLA-B and HLA-C ligands," *Journal of Immunology*, vol. 179, no. 9, pp. 5977–5989, 2007.

[79] S. Giebel, F. Locatelli, T. Lamparelli et al., "Survival advantage with KIR ligand incompatibility in hematopoietic stem cell transplantation from unrelated donors," *Blood*, vol. 102, no. 3, pp. 814–819, 2003.

[80] L. Ruggeri, A. Mancusi, K. Perruccio, E. Burchielli, M. F. Martelli, and A. Velardi, "Natural killer cell alloreactivity for leukemia therapy," *Journal of Immunotherapy*, vol. 28, no. 3, pp. 175–182, 2005.

[81] D. W. Beelen, H. D. Ottinger, S. Ferencik et al., "Genotypic inhibitory killer immunoglobulin-like receptor ligand incompatibility enhances the long-term antileukemic effect of unmodified allogeneic hematopoietic stem cell transplantation in patients with myeloid leukemias," *Blood*, vol. 105, no. 6, pp. 2594–2600, 2005.

[82] T. Kawase, K. Matsuo, K. Kashiwase et al., "HLA mismatch combinations associated with decreased risk of relapse: implications for the molecular mechanism," *Blood*, vol. 113, no. 12, pp. 2851–2858, 2009.

[83] D. Cho, D. R. Shook, N. Shimasaki, Y.-H. Chang, H. Fujisaki, and D. Campana, "Cytotoxicity of activated natural killer cells against pediatric solid tumors," *Clinical Cancer Research*, vol. 16, no. 15, pp. 3901–3909, 2010.

[84] S. Arai, R. Meagher, M. Swearingen et al., "Infusion of the allogeneic cell line NK-92 in patients with advanced renal cell cancer or melanoma: a phase I trial," *Cytotherapy*, vol. 10, no. 6, pp. 625–632, 2008.

[85] E. G. Iliopoulou, P. Kountourakis, M. V. Karamouzis et al., "A phase i trial of adoptive transfer of allogeneic natural killer cells in patients with advanced non-small cell lung cancer,"

Cancer Immunology, Immunotherapy, vol. 59, no. 12, pp. 1781–1789, 2010.

[86] M. A. Geller, S. Cooley, P. L. Judson et al., "A phase II study of allogeneic natural killer cell therapy to treat patients with recurrent ovarian and breast cancer," *Cytotherapy*, vol. 13, no. 1, pp. 98–107, 2010.

[87] L. Barkholt, E. Alici, R. Conrad et al., "Safety analysis of ex vivo-expanded NK and NK-like T cells administered to cancer patients: a Phase I clinical study," *Immunotherapy*, vol. 1, no. 5, pp. 753–764, 2009.

[88] V. Decot, L. Voillard, V. Latger-Cannard et al., "Natural-killer cell amplification for adoptive leukemia relapse immunotherapy: comparison of three cytokines, IL-2, IL-15, or IL-7 and impact on NKG2D, KIR2DL1, and KIR2DL2 expression," *Experimental Hematology*, vol. 38, no. 5, pp. 351–362, 2010.

[89] Y. Z. Jiang, A. J. Barrett, J. M. Goldman, and D. A. Mavroudis, "Association of natural killer cell immune recovery with a graft-versus-leukemia effect independent of graft-versus-host disease following allogeneic bone marrow transplantation," *Annals of Hematology*, vol. 74, no. 1, pp. 1–6, 1997.

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Review Article

Extranodal NK/T-Cell Lymphoma: Toward the Identification of Clinical Molecular Targets

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Extranodal natural killer (NK)/T-cell lymphoma of nasal type (NKTCL) is a malignant disorder of cytotoxic lymphocytes of NK or more rarely T cells associated with clonal Epstein-Barr virus infection. Extranodal NKTCL is rare in Western countries, but in Asia and Central and South America it can account for up to 10% of non-Hodgkin's lymphomas. It is an aggressive neoplasm with very poor prognosis. Although the pathogenesis of extranodal NKTCL remains poorly understood, some insights have been gained in the recent years, especially from genome-wide studies. Based on our own experience and knowledge of the literature, we here review some of the genomic and functional pathway alterations observed in NKTCL that could provide a rationale for the development of innovative therapeutic strategies.

1. Introduction

The term extranodal natural killer/T-cell lymphoma (NKTCL) refers to a group of clonal proliferations of cytotoxic lymphocytes of natural killer (NK) or, more rarely, T-cell types, with peculiar clinicopathologic features, arising mainly as tumors or destructive lesions in the nasal cavity, maxillary sinuses, or palate [1]. More rarely, extranodal NKTCL may present in other extranodal sites such as skin, testis, lung, or gastrointestinal tract and tend to have a more adverse clinical outcome [2–4]. This is particularly true when one defines nonnasal cases as extra upper aerodigestive tract cases as in the study of Lee et al. which reports survival rates of 20% versus 54% for the patients with nasal and upper airway region localizations [4]. However, as noted by several authors, many nonnasal NKTCL might represent disseminated nasal NKTCL, knowing that such dissemination can occur early in the clinical course of the disease and toward sites that are localizations where nasal NKTCL will metastasize to. Besides the more adverse clinical features, for which the underlying mechanisms need to be defined, there are no significant differences in age, gender, ethnicity, bone marrow involvement, hemophagocytosis, or immunophenotypic profiles between nasal and nonnasal NKTCL. Very rare cases with primary lymph node involvement have also been described [5]. Extranodal NKTCL shows a wide cytological spectrum and is characterized by frequent features of angioinvasion and angiocentrism, which often result in coagulative necrosis. Typically, tumor cells express cytoplasmic CD3ε, CD2, CD56, lack CD5, CD4, and CD8, and have an activated cytotoxic immunophenotype with expression of perforin,

granzyme B, and TiA1. The cell of origin of these tumors has been debated. Indeed, the identification of CD3 expression in tumor cells in necrotic lesions has led to the terminology of angiocentric T-cell lymphoma [6–8] adapted in the REAL classification [9], before it was demonstrated that tumor cells express several chains of the CD3 complex in the cytoplasm, but lack T-cell receptors (TCR) and have TCR genes in a germline configuration, consistent with an NK-cell origin [10, 11]. If the majority of NKTCL are likely to originate from mature NK cells, a small proportion of cases, with expression of $\gamma\delta$ or $\alpha\beta$ TCR appear to derive from cytotoxic T lymphocytes, reflected in the "NK/T" cell terminology.

Extranodal NKTCL represents the major group of mature NK cell neoplasms in the recently revised WHO classification of hematolymphoid tumors, which also include the aggressive NK cell leukemia (ANKL) and a provisional group of chronic NK-cell lymphoproliferative disorder of uncertain malignant potential, most likely related to T-cell large granular lymphomas [1]. Importantly, both NKTCL and ANKL are Epstein-Barr virus- (EBV-) associated neoplasms as the virus is found in their tumor cells [12, 13]. Although the precise role of the virus in the etiology of the disease is poorly understood, the study of EBV gene polymorphism has shown that tumor cells are clonally infected as opposed to normal nasal tissues [14, 15]. Circulating EBV viral load is an important prognostic factor, and plasma EBV DNA levels can also be used for disease monitoring [16]. In this respect, the incidence of NKTCL parallels the geographic distribution of EBV infection with prevalence in the Asian and Central and South American populations, where it can account for up to 10% of non-Hodgkin's lymphomas [17–19].

Despite a localized presentation in most patients, extranodal NKTCL is an aggressive disease with poor prognosis. The 5-year survival rate is less than 50%. In the absence of effective treatment, the median survival for advanced-stage disease is only 6–12 months [19–22]. The retrospective International Peripheral T-cell Lymphoma project recently reported a median overall survival of 7.8 months for NKTCL, corresponding to the poorest survival among all T-cell lymphoma entities [2]. Therefore, despite progress with combined field radiotherapy and chemotherapy, autologous bone marrow transplantation and the promising effect of L-asparaginase treatment in relapsed cases [16, 23], NKTCL remains difficult to cure, and the need for alternative therapeutic strategies has prompted researchers to explore oncogenic pathways involved, to provide new molecular targets. This review will focus on the these potential molecular pathways that have been implicated in the physiopathology of NKTCL, in particular through the lights shed by several recently reported genome-wide profiling studies [24–30].

2. EBV Infection and Viral Protein Expression

Several lines of evidence point at EBV as a major player in the pathogenesis of NKTCL. First of all, when dealing with an EBV-associated malignancy, one can think of NKTCL as a potentially highly immunogenic lymphoma that could benefit from cellular immunotherapies targeting the viral antigens as in posttransplant B-cell lymphoproliferative disorders [31]. These B-cell proliferations, like the *in vitro* infected B-cell-derived lymphoblastoid cell line (LCL), express the full spectrum of EBV latent proteins (latency III). The latent phase consists of the maintenance of the EBV genome as a circular episome that is replicated by the cellular DNA polymerase. Latent type III infection is associated with the expression of six EBV-encoded nuclear antigens (EBNA): EBNA1, EBNA2, EBNA2A, EBNA3B, EBNA3C, and EBNA leader protein (EBNA-LP); three cell surface proteins: latent membrane protein (LMP) 1, LMP2A, and LMP2B; two EBV-encoded RNAs: EBER1 and EBER2. Among these proteins, the EBNA3s are believed to be particularly immunogenic for the generation of specific cytotoxic T lymphocytes (CTL). However, similarly to Hodgkin's lymphoma, NKTCL is believed to be in latency II, with a more restricted pattern of viral proteins expressed, limited to EBNA1, LMP1, and LMP2 [32]. EBNA1 is required for the maintenance of viral episomes and expressed in all latency phases. LMP1 is the main transforming protein of EBV. It is essential for EBV-induced B-cell transformation *in vitro*, acting like a constitutively activated tumor necrosis factor receptor family member, activating NF-κB pathway and promoting cell survival. LMP2A and LMP2B are generated by alternative splicing and, although not essential for B-cell transformation, can also promote proliferation and survival. However, LMP2 has only been demonstrated at the transcriptional level in NKTCL. LMP1 expression is also variable, and when expressed it is often limited to a subpopulation of tumor cells, possibly under the pressure of host immune surveillance. Despite these challenging conditions, attempts have been made to generate *in vitro* LMP2-specific CTL using antigen-presenting cells overexpressing LMP2, to treat Hodgkin's lymphoma and NKTCL patients [33]. The cytotoxic activity of these LMP2-specific CTL have been confirmed against NKTCL-derived cell line, which led to the discovery of a unique alternative transcript of LMP2 (LMP2-TR) that uses a new promoter located in the terminal repeat region of the episomal EBV, not expressed in B-LCL [34]. LMP2-TR may constitute a promising target for adoptive cellular immunotherapy in NKTCL.

EBV is a B-lymphotropic virus, infecting B cells through interaction with its cellular receptor CD21, and establishing a lifelong infection in more than 90% of the human adult population. Although the precise mechanism involved in NK- or T-cell infection is unknown, passive acquisition of CD21 in the NK/T-cells by membrane fragment exchange during cytotoxic interaction, a process known as trogocytosis, has been proposed [35, 36]. Chronic active EBV infection (CAEBV) was first recognized as severe illness related to chronic or persistent EBV infection [37, 38]. CAEBV is a life-threatening disease characterized by infectious mononucleosis-like symptoms with virus-associated hemophagocytic syndrome. Like in NKTCL, clonal proliferations of EBV-infected NK- or T-cells are seen in patients with CAEBV, making this disease more likely a chronic lymphoproliferative disorder or a premalignant stage as some CAEBV patients can actually develop subsequent NKTCL [39]. Whether the EBV-host interaction may play a role

in the development of NKTCL is still a pending question. Performing comparison of EBV and cellular gene expression profiling in NKTCL- and CAEBV-derived cell lines, Zhang et al. reported the transcription of lytic phase genes such as *BZLF1*, *BARF1*, *BFLF2,* and *BDLF3*, despite the absence of virion DNA production and cellular gene signature implicating mainly cell cycle and apoptosis-related genes such as *TNFRSF10D, CDK2, Hsp90, IL12A, and PDCD4* [30]. Beside the expression of latency II genes, expression in NK/T-cell lines, of lytic phase genes (such as *BZLF1*) capable to interact with multiple cell cycle control proteins is intriguing. EBV may exert oncogenic effects through the production of cytokines such as Interleukin 9 (IL-9) [26] and IL-10 [40]. EBV-induced production of IP10/MIP2 chemokines may contribute to vascular damage and necrosis [41]. Obviously, the way viral gene products interact with cellular host metabolism needs to be clarified and may lead to novel targets for therapies.

3. NK Cell Antigens and Cytotoxic Activity

Irrespective of their NK, $\alpha\beta$-, or $\gamma\delta$-T-cell lineage, NKTCL is a lymphoma of activated cytotoxic lymphocytes as evidenced by the expression of cytotoxic molecules such as perforin, granzyme B, and TIA1 [42]. Whereas T cells use their TCR for antigen recognition and control of their specific cytotoxic activity, NK cells use MHC class I-specific NK cell receptors (NKR) for this purpose [43]. NK cells are part of the innate immune system involved in the immunosurveillance [44]. Engagement of inhibitory NKR by self-MHC class I on potential target cells inhibits cytotoxicity whereas loss of MHC class I expression due to transformation or viral infection, results in a loss of this inhibition. There are two types of NKR: the killer immunoglobulin-like receptors (KIR) and the killer cell lectin-like receptors (KLR or CD94/NKG2 complexes). KLR as well as other NK cell markers like CD56 can also be expressed by $\alpha\beta$- or $\gamma\delta$-cytotoxic T cells. The *KIR* locus located on human chromosome 19, exhibits a substantial haplotypic and allelic diversity [45, 46]. The KIR haplotypes have been separated into two groups, containing from 7 to 12 genes [47]. The expression of KIR at the cell surface is clonally distributed, meaning that a clonal NK/T-cell population is expected to express a restricted KIR repertoire as shown in NKTCL [48–50]. Another example of the interest of KIR in malignant lymphomas is given by KIR3DL2 that proved to be a valuable tool in the diagnosis, and perhaps a clinical target, of Sézary syndrome, a cutaneous $CD4^+$ T-cell lymphoma with systemic dissemination [51–53]. KLR are C-type lectin receptors that precede the expression of KIR during NK cell development. They consist of molecules of the NKG2 family such as NKG2A (KLRC1), NKG2C (KLRC2), or NKG2E (KLRC3), form heterodimer with earlier expressed CD94 at the surface of maturing NK cells [54]. In addition to immature NK cells, a subset of mature NK cells also lack CD94/NKG2 expression as a consequence of the clonal diversification phenomenon. Interestingly, expression of CD94 has been associated with a better prognosis in NKTCL [55]. Although NK activity was identified from the initial description of ANKL [56, 57], the cytotoxic activity of the NKTCL tumor cells has not been particularly investigated due to the difficulty to isolate these cells.

4. Survival/Apoptosis

The mechanisms by which NKTCL cells survive and escape immune surveillance are unknown. One way for an NKTCL tumor cell to escape anti-EBV immunity is to lower its expression of EBV antigens, particularly LMPs proteins. It is also known that cytotoxic effector cells have a proper way to resist to their own cytotoxic agents [58]. The main cytotoxic pathway used by NK and CTL involves the release from specialized granules of cytotoxic molecules including perforin and granzymes, triggering a process leading to DNA fragmentation and apoptosis. In particular, granzyme B (GZMB), by activating directly or indirectly caspase-3, leads to rapid execution of apoptosis. SERPINB9 (also known as PI9) is a GZMB-specific serine protease inhibitor that can protect effector cells from their own cytotoxic activity. It has been shown that SERPINB9 can make hepatocytes resistant to lysis by CTL or NK cells [59] and may participate to the mechanism for tumor escape in NKTCL [60]. Interestingly, its loss of expression was described as a poor prognostic factor in a study of 48 NKTCL patients [20].

Although it seems that tumor cells are resistant to the killing via GZMB, NKTCL are frequently associated with zonal tumor cell death, tissue necrosis, and vascular damage. The production of chemokines like CXCL9 (Mig) and CXCL10 (IP10), in response to interferon γ released by activated tumor cells, may contribute to the observed damages [41, 61]. Although Fas (CD95) as well as Fas ligand (FASL/CD95L) are frequently well expressed in NKTCL cells, mutations of the *FAS* gene are observed in 50 to 60% of cases, most likely contributing to resistance to apoptosis [62]. Indeed, most of the *FAS* gene mutations are frameshift mutations arising in the death domain, leading to mutated proteins on the cell surface, unable to transduce the apoptotic signal [63]. High expression of FASL is also a way for tumor cells to evade immune response by deleting Fas-expressing infiltrating T cells. In line with these observations, NKTCL has long been known as resistant to combination chemotherapy. This resistance is also complicated by the high expression of p-glycoprotein, encoded by multidrug resistance gene 1 (*MDR1*) that acts as an energy-dependent efflux pump for various drugs [64].

Very recently, survivin (BIRC5), an inhibitor of apoptosis frequently involved in tumor oncogenesis, was found overexpressed in NKTCL [28]. The authors proposed that survivin could be a useful therapeutic target in NKTCL. In *in vitro* studies, they showed that Terameprocol, a survivin inhibitor, could downregulate survivin expression and induced apoptosis in NK cell lines.

5. Cytogenetic Analysis

Lymphomas arise from clonal expansion of transformed lymphoid cells through the accumulation of genetic lesions.

Currently, no genetic abnormalities specific for NKTCL have been identified. Cytogenetic analyses of NKTCL are difficult because of necrosis, small-size samples, and contamination by inflammatory reactive cells. Despite these difficulties, several studies using comparative genomic hybridization (CGH) and loss of heterozygosity (LOH) techniques have been reported [65–70]. Altogether, these studies identified gains at chromosomes 1p, 6p, 11q, 12q, 17q, 20q, Xp, and losses at 6q, 11q 13q, and 17p. In particular, the most frequent deletion was observed at 6q21–25 in all but one study. Sun et al. tried to define a minimal tumor suppressor gene-containing region involved in del6q25 and identified a 2.6 Mb interval located between *TIAM2* and *SNX9* genes [69]. More recently, progress in the DNA chip technology allowed the development of genome-wide array-based CGH (aCGH) giving access to better resolutions. The first report, from Nakashima et al., performed with homemade arrays with 1.35 Mb resolution, studied 10 of ANKL and 17 NKTCL cases and reported differences in their genomic alteration patterns (see Table 1) [27]. Loss of 7p (40%) and 17p13.1 (40%) and gain of 1q (43%) occurred more frequently in ANKL whereas loss of 6q did not. On the other hand, loss of 6q21–q22.1 (35%), 6q22.33–q23.2 (47%), 6q25.3 (29%), and 6q26-q27 (35%) were more frequent in NKTCL. Other alterations in NKTCL compared to ANKL include gain in 2q and loss of 1p36.23–p36.33, 2p16.1–p16.3, 4q12, 4q31.3–q32.1, 5p14.1–p14.3, 5q34–q35.3, and 11q22.3–q23.3. Two recent studies extended these findings by comparing submegabase resolution aCGH and gene expression profiling on NK cell lines as well as NKTCL tumor samples [24, 71]. These two studies emphasize recurrent genomic abnormalities observed in about half of the patients: gain of 1q21–q44, and losses of 17p11.2–p13.3 and 6q21. In particular, this region of del6q21 contains four candidate tumor suppressor genes, *PRDM1*, *ATG5*, *AIM1,* and *HACE1*, which decreased expression was confirmed at the RNA level. In their study, Iqbal et al. found mutations and methylation in *PRDM1*, *ATG5*, and *AIM1* in NKTCL cell lines [71]. *ATG5* is part of the autophagy pathway implicated both in apoptosis and in the maintenance of energy homeostasis during starvation [72]. The role of autophagy in oncogenesis is, however, the object of controversy [73]. *AIM1* which stands for "absent in melanoma-1" is still poorly characterized but was reported as a good tumor suppressor gene candidate in malignant melanoma, exerting its effects through interactions with the cytoskeleton [74]. *PRDM1* is a transcriptional regulator, initially described as a suppressor of beta-interferon gene expression, that is associated with the terminal differentiation of B lymphocytes and T-cell homeostasis and function [75, 76]. It was found mutated in some diffuse large B-cell lymphomas, and it was concluded that its inactivation contributes to lymphomagenesis by blocking differentiation of postgerminal center B cells to plasma cells [77]. Loss-of-function due to mutations as well as transcriptional inhibition by DNA methylation observed in NK cell lines, suggest a tumor suppressor role of PRDM1 in NKTCL [71].

HACE1 is another candidate tumor suppressor gene [24, 90]. Characterization of the chromosome 6q21 breakpoint, frequently involved in sporadic Wilm's tumors, led to the identification of the *HACE1* gene [91]. It encodes a member of the HECT family of E3 ubiquitin ligases that tag specific target proteins to degradation by the proteasome or to control their subcellular localization [85]. Because of their involvement in controlling crucial signaling pathways, E3 ubiquitin ligases appear as important regulators of cancer development and therapy. It is downregulated in multiple human cancers and maps to a prominent tumor-suppressor region of LOH in many tumors including lymphomas. $HACE1^{-/-}$ mice are also prone to spontaneous development of various types of cancers [90]. Our integrated genomic and transcriptomic analysis of NKTCL tumor samples and cell lines has shown downexpression of *HACE1* gene irrespective of the presence of del6q21 alteration [24]. In addition, we could show by methylation-specific PCR and product sequencing (data not shown) that hypermethylation of CpG-177 island located directly upstream of *HACE1* locus, is responsible of the silencing of the diploid gene or the remaining allele. Although the mechanism of antitumor action of HACE1 is still unclear, it has been shown that overexpression of HACE1 in the 293T cell line inhibits cell proliferation via the degradation of phosphorylated cyclin D1 [90]. This suggests a possible mechanism for HACE1 to regulate cell cycle exit by reducing cyclin D1 levels. Existence of other HACE1 targets is possible as suggested by the recent identification of retinoic acid receptor beta 3 as an HACE1 interacting protein [92].

6. Cell Signaling Pathways

Although the anomaly of signaling pathways involved in the pathogenesis of NKTCL is not deciphered, progress has been made in the recent years that will help to define candidate therapeutic targets.

6.1. Jak-Stat Pathways. Signal transducers and activators of transcription, STATs, are transcription factors activated in response to cytokines or growth factors [80]. The activation of STAT signaling pathways requires tyrosine phosphorylation of the STAT proteins that results from their association with growth factor receptors having intrinsic tyrosine kinase activity, or through recruitment of members of the Janus kinase (JAK) family to activated surface receptors. A direct link between STAT signaling and oncogenesis was established when it was shown that constitutive STAT activation by oncogenic tyrosine kinases from *Src* or *Abl* families, directly participates to cell transformation [93, 94]. Accordingly, a large number of tumors, both in primary cells as well as in tumor-derived cell lines, display constitutive activation of STAT factors. In particular, STAT3 role in oncogenesis is well documented in anaplastic large cell lymphoma where STAT3 activation was shown to provide growth advantage and resistance to apoptosis of ALK^{+} tumor cells [81, 95, 96]. In NKTCL, STAT3 was found constitutively activated, by phosphorylation on Y705, and localized in the nucleus in 90% of the patients studied [24, 82]. Inhibition of endogenous

Table 1: Recurrent genomic alterations in extranodal NKTCL from published data.

Chromosomal location	Frequency (%)						
	Huang et al. 2010 [24]	Iqbal et al. 2009 [71]	Nakashim et al. 2005 [27]	Yoon and Ko 2003 [70]	Ko et al. 2001 [66]	Siu et al. 2000 [67]	Siu et al. 1999 [68]
Gains							
1q21–q44	50	50	50				20
2q13-q14		26	24		29		
2q31.1–q32.2		20	24		43		
6p25–p11.1	40	25	40				
7q11.2–q34	50	32	24				20
7q35-q36	60	40					40
17q21.1	20	50			29		40
20pter-qter	30	45					50
Losses							
6q16–q25	40	50	38	80		49	25
11q23.1	30	13	29			31	20
11q24-q25	40	25					20
13q14.11	30	27	25			14	60
17p13.3	40	45	40		43	40	20

Table 2: Potential Therapeutic targets for extranodal NKTCL.

Pathways	Genes	Expression	Chromosomal location	References
EBV	LMP2-TR	Up	N.A.	[32]
Survival/apoptosis				
	CCND3	Down	6p21.1	[69]
	SERPINB9	Down	6p25	[19, 57]
	FASL	Up	1q23	[60, 78]
	TNFAIP3	Down	6q23	[69, 79]
Cell signaling pathways				
JAK-STAT	STAT3	Up	17q21	[80, 81]
	JAK2	Up	9p24	[69, 81]
	IL10	Up	1q31-q32	[81, 82]
AKT	AKT1/2/3	Up	14q32.3/19q13.1/1q44	[69, 70]
NOTCH	NOTCH1	Up	9q34.3	[24, 83]
WNT	β-CATENIN	Up	3p22–p21.3	[24, 69]
PDGF	PDGFRA	Up	4q11–q13	[69]
	PDGFA/B	Up	7p22/22q12.3–q13.1	[69]
Angiogenesis				
	HGFR (MET)	Up	7q31	[78]
	VEGFR2	Up	4q12	[24, 69]
	VEGFA	Up	6q12	[24, 69]
	HIF1α	Up	14q21–q24	[24, 70, 84]
Tumor suppressor genes				
del6q21	PRDM1	Down	6q21–q22.1	[70]
	ATG5	Down	6q21	[70]
	AIM1	Down	6q21	[70]
	HACE1	Down	6q21	[69, 85]
Other pathways/genes				
	TP53	Down	17p13.1	[86, 87]
	TP73	Down	1p36.3	[88]
	AURKA	Up	20q13	[24, 89]

N.A.: not applicable

activated STAT3 in NKTCL-derived cell lines, using transducible dominant-negative STAT3, leads to growth arrest and apoptosis inhibition [82]. These results are consistent with the known targets of STAT3 transcriptional activities that include survivin, cyclin D1, BCL-X_L, cMYC, vascular growth factor (VEGF), IL-10, and IL-6 [97]. Importantly, STAT3 activation promotes the production of immunosuppressive factors not only by the tumor cells but also by cells of the microenvironment through mediators such as VEGF or IL-10, activating STAT3 in these bystander cells that in turn restrains antitumor immune response [84, 98]. Targeting STAT3 and/or JAK2 for cancer immunotherapy may therefore be promising.

6.2. Angiogenesis Pathway. NKTCL is characterized by angiocentric growth and vascular damages with invasion of vessel walls by lymphoma cells. In this context, a search for genes associated with angiogenesis seems essential. The recent gene expression profiling studies have recorded overexpression of genes related to angiogenesis in NKTCL as compared to other peripheral T cell lymphomas or normal NK cells [24, 25]. Among those, one finds *VEGFA* an important downstream product of STAT3, and its receptor *VERGFR2* (*KDR*), both genes are upregulated, a finding confirmed by the immunohistochemical detection of VEGFα and its receptor in tumor cells. As mentioned previously, the production of VEGF by tumor cells not only may be used for autocrine growth and survival but also to maintain an immunosuppressive microenvironment by activating the STAT3 pathway. Vascular destruction observed in nasal type NKTCL results in hypoxia and the transcription activation of HIFα (hypoxia-inducible factor 1), a transcriptional activator that stimulates the expression of a number of genes implicated in angiogenesis (including *VEGFA* and *VEGFR2*) and in oxygen homeostasis [78]. Also observed is the upregulation of another important gene in angiogenesis, the *c-MET* proto-oncogene that encodes the tyrosine kinase cell surface receptor for the hepatocyte growth factor (HGF). HGF is a pleiotropic cytokine acting as an antiapoptotic, a promigratory, and a proliferating factor for a number of tissues. Interestingly, MET may be implicated in the prevention of FAS-mediated apoptosis of the FAS-expressing NKTCL tumors cells. This relies on the YLGA aminoacid motif located near the N-terminal region of MET that specifically binds to the extracellular portion of FAS and acts as a FASL antagonist and inhibitor of FAS trimerization [99].

6.3. PDGFR Pathway. The platelet-derived growth factor receptor- (PDGFR-) signaling pathway was also found activated in NKTCL cells [24]. The overexpression of PDGFRA mRNA in NKTCL compared to normal NK cells in microarray data is confirmed by the expression of PDGFRα and its phosphorylated form in primary tumors, shown by immunohistochemistry. Neither genomic abnormalities in the *PDGFRA* locus in 4q11–q13 nor mutations in the coding sequence or anomalies in the promoter region could be evidenced. The mechanism responsible for the activation of the PDGFR remains therefore unclear, but like in PTCL NOS (PTCL not otherwise specified) [83], an autocrine feedback loop may explain the presence of the phosphorylated PDGFRα observed in all the 13 NKTCL cases examined. The potential of PDGF signaling pathway in NKTCL cell proliferation is illustrated by the dramatic dose-dependent inhibition of MEC04 cell growth observed in cultures performed the presence of imatinib mesylate [24].

6.4. Other Signaling Pathways. Pathways enriched in the gene signature of NKTCL include those of the NOTCH, WNT, and NFκB signaling pathways [24, 25, 30]. However, and particularly in this tumor where necrosis is associated with strong infiltration of mesenchymal and lymphoid cells, it is sometimes difficult to attribute the differentially expressed genes to the tumor cells rather than to the microenvironment cells. In addition, differential expression needs confirmation in terms of biochemical activation. In particular, activation of the WNT pathway, comprising genes involved during the development and in oncogenesis, can be evidenced by the nuclear translocation of β-catenin. However, no nuclear expression of β-catenin could be observed in the neoplastic cells in our study by Huang et al. [24], making the targeting of WNT pathway of poor interest for therapeutic purpose. On the other hand, activation of the NOTCH pathway has been validated by Iqbal et al. [25]. NOTCH signals regulate development and differentiation of adult self-renewing cells, including T cells. Gamma-secretase is a critical component of the NOTCH signal transduction pathway [100]. Showing that inhibitors of γ-secretase can block the proliferation and survival of NK cell lines *in vitro,* was used for validation of NOTCH implication. Interestingly, *PTEN* is a tumor suppressor gene encoding a lipid phosphatase that antagonizes the activation of the PI3K/AKT pathway. NOTCH1 controls a transcriptional network that leads to activation of PI3K/AKT signaling and downmodulation of PTEN expression. NOTCH signaling and PI3K/AKT pathway act synergistically to maintain oncogenic activity in T-cell acute lymphoblastic leukemia [101]. PI3K/AKT was found activated in microarray analysis of NKTCL, and nuclear expression of phosphorylated-AKT was found in the nucleus of most NKTCL samples. NFκB is a master regulator that controls the expression of a number of genes. Because EBV is known to activate NFκB through LMP-1 and/or TRAF signaling [79], upregulation of NFκB pathway in NKTCL versus normal NK cells is not unexpected. Signaling by the transcription factor NFκB involves its release from its inhibitor IκB, followed by its translocation into the nucleus. Nuclear detection by immunohistochemistry of RelA, the protein constituting the most abundant form of NFκB, further supports the activation of this pathway in NKTCL. Interestingly, the tumor necrosis factor-α-induced protein gene, *TNFAIP3*, an inhibitor of NFκB activity, was also found downregulated in NKTCL. This gene is located in 6q23, the region of recurrent loss discussed above, and loss of function of TNFAIP3 have also been reported in Hodgkin's lymphoma and other B-cell lymphomas [86, 102]. Surprisingly, the NFκB pathway genes were not enriched in the NKTCL gene signature in the recent study of Iqbal et al. [25]. Questions

remain, therefore, to clarify the value of this pathway to identify therapeutic targets for NKTCL.

7. Other Pathways and Candidate Target Genes

Many other genes are found differentially expressed or targets for mutations or methylations in NKTCL when compared to normal NK cells (see Table 2). The list includes a number of oncogenes or tumor suppressor genes associated with many other tumors. *TP53* is a well-known tumor suppressor gene that causes cells with damaged DNA to arrest at G1 phase of the cell cycle. TP53 is mutated in many cancers, and the frequency of NKTCL-mutated cases varies from 20 to 60%. *TP53* mutated cases have been associated with more advanced cases, suggesting that *TP53* mutation represents a secondary oncogenic event rather than a triggering mechanism for the development of NKTCL [87]. Nevertheless, *TP53* is found underexpressed in NKTCL transcriptomic analyses, and the observation that EBNA1 promotes TP53 degradation [88] has to be considered to make TP53 pathway a possible target for NKTCL therapy. *TP73* belongs to the family of p53-related proteins, which is involved in cell cycle arrest and apoptosis. Methylation of *TP73* has been reported in 94% of NKTCL and has been proposed as a biomarker to detect NKTCL involvement and metastasis [103]. *MYC* family genes encode multifunctional nuclear phosphoproteins that function as transcription factors to regulate expression of genes involved in cell cycle progression such as *CCNA2*, *CDKN1A*, and *CDKN2B*. Although frequent in hematopoietic and solid tumors, mutations, amplification, and translocation of this oncogene have not been found in NKTCL. However, *MYC* expression appears to be upregulated in the gene signature of NKTCL.

Adhesion and cell-to-cell interaction molecules may play an important role in tumor growth and homing in view of the particular tropism of NKTCL for extranodal sites. The molecular signatures reported for NKTCL in comparison with normal NK cells include overexpression of genes of the cadherin family (CDH1), of the integrin family (VCAM1, ITGA7, ITGA9, and ITGB4), and of the sialoadhesin family (SIGLEC1, SIGLEC 9, SIGLEC11, and SIGLECP3). Protocadherin 15 (PCDH15) was shown to be expressed ectopically by NKTCL cells and NKTCL-derived cell lines [104], and confirmed in gene expression profiling [24]. PCDH15, normally absent in the hematopoietic tissues, may be used by NK tumor cells to escape immunosurveillance, like CDH1 recognized by the KLRG1 inhibitory NK receptor [105].

Recently, AURKA, the Aurora kinase A or ST6, was reported as another potential target for NKTCL [25]. AURKA is a mitotic centrosomal protein kinase that controls chromosome segregation during mitosis [89, 106]. AURKA is activated by phosphorylation and could be detected in all human NK cell lines tested, validating its transcriptomic overexpression observed in NKTCL versus normal NK cells. In many other cancers, AURKA upregulation results in a phenotype characteristic of loss-of-function mutations of *TP53*. Katayama et al. showed that TP53 is phosphorylated by AURKA, inducing its ubiquitination by MDM2 and its proteasomal degradation [107]. Thus, AURKA is a key regulator of TP53 pathway, and the AURKA inhibitor MK8751 induced cell cycle arrest and apoptosis in human cell lines, including NKTCL-derived cell lines [25]. AURKA represent, therefore, an interesting potential therapeutic target for NKTCL.

8. Conclusion

ANKL and NKTCL are EBV-associated malignancies of cytotoxic lymphocytes with poor prognosis and lack of efficient therapies. Their aggressive behavior and poor response to chemotherapy make of paramount importance the search for novel therapeutic targets in the treatment of these lymphomas. NKTCL is a rare disease although more frequent in Asia and South America, and the insufficient supply of tumor samples limits the development of an intense research in that field. In this context, malignant NK/T-cell lines represent valuable tools to study these pathologies [108]. Genome-wide studies have generated new data improving our understanding of the disease. Indeed, candidate tumor suppressor genes, such as *PRDMI*, *ATG5*, *AIM1*, or *HACE1*, which can be inactivated by deletion and by methylation, have been identified. The molecular signature of NKTCL is distinct from that of other T-cell lymphoma subtypes. Compared to normal NK cells, NKTCL is characterized by activation of several pathways—PDGFRA, VEGFR, AKT, JAK-STAT, and NOTCH—which might represent targets for novel therapeutic options. Before settingup clinical trials, extensive experimental validation of the potential target will have to be undertaken using the available models. In this respect, the study of tumor engrafting and development in immunocompromised mice injected with NKTCL cell lines or tumor fragments [82, 109] can be valuable models to test new potential drugs and their *in vivo* activity. One can hope that the efforts of the researchers working to understand this pathology will soon lead to progress in the treatment of these lymphomas.

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References

[1] S. Swerdlow, E. Campo, N. Harris, E. Jaffe, and S. H. S. Pileri, *WHO Classification of Tumors of the Haematopoietic and Lymphoid Tissues*, International Agency of Research on Cancer (IARC), Lyon, France, 2008.

[2] W. Y. Au, D. D. Weisenburger, T. Intragumtornchai et al., "Clinical differences between nasal and extranasal natural killer/T-cell lymphoma: a study of 136 cases from the International Peripheral T-Cell Lymphoma Project," *Blood*, vol. 113, no. 17, pp. 3931–3937, 2009.

[3] J. K. C. Chan, V. C. Sin, K. F. Wong et al., "Nonnasal lymphoma expressing the natural killer cell marker CD56:

a clinicopathologic study of 49 cases of an uncommon aggressive neoplasm," *Blood*, vol. 89, no. 12, pp. 4501–4513, 1997.

[4] J. Lee, H. P. Yeon, S. K. Won et al., "Extranodal nasal type NK/T-cell Lymphoma: elucidating clinical prognostic factors for risk-based stratification of therapy," *European Journal of Cancer*, vol. 41, no. 10, pp. 1402–1408, 2005.

[5] E. Takahashi, N. Asano, C. Li et al., "Nodal T/NK-cell lymphoma of nasal type: a clinicopathological study of six cases," *Histopathology*, vol. 52, no. 5, pp. 585–596, 2008.

[6] A. Aviles, L. Rodriguez, R. Guzman, A. Talavera, E. L. Garcia, and J. C. Diaz-Maqueo, "Angiocentric T-cell lymphoma of the nose, paranasal sinuses and hard palate," *Hematological Oncology*, vol. 10, no. 3-4, pp. 141–147, 1992.

[7] J. K. C. Chan, C. S. Ng, P. K. Hui, S. T. H. Lo, and W. H. Lau, "Angiocentric T-cell lymphoma of the skin: an aggressive lymphoma distinct from mycosis fungoides," *American Journal of Surgical Pathology*, vol. 12, no. 11, pp. 861–876, 1988.

[8] Y. Ishii, N. Yamanaka, and K. Ogawa, "Nasal T-cell lymphoma as a type of so-called "lethal midline granuloma"," *Cancer*, vol. 50, no. 11, pp. 2336–2344, 1982.

[9] N. L. Harris, E. S. Jaffe, H. Stein et al., "A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group," *Blood*, vol. 84, no. 5, pp. 1361–1392, 1994.

[10] J. F. Emile, M. L. Boulland, C. Haioun et al., "CD5-CD56+ T-cell receptor silent peripheral T-cell lymphomas are natural killer cell lymphomas," *Blood*, vol. 87, no. 4, pp. 1466–1473, 1996.

[11] J. Suzumiya, M. Takeshita, N. Kimura et al., "Expression of adult and fetal natural killer cell markers in sinonasal lymphomas," *Blood*, vol. 83, no. 8, pp. 2255–2260, 1994.

[12] P. Kanavaros, M. C. Lescs, J. Briere et al., "Nasal T-cell lymphoma: a clinicopathologic entity associated with peculiar phenotype and with Epstein-Barr virus," *Blood*, vol. 81, no. 10, pp. 2688–2695, 1993.

[13] A. K. Ruskova, R. Thula, and G. T. C. Chan, "Aggressive natural killer-cell leukemia: report of five cases and review of the literature," *Leukemia and Lymphoma*, vol. 45, no. 12, pp. 2427–2438, 2004.

[14] A. K. S. Chiang, K. Y. Wong, A. C. T. Liang, and G. Srivastava, "Comparative analysis of Epstein-Barr virus gene polymorphisms in nasal T/NK-cell lymphomas and normal nasal tissues: implications on virus strain selection in malignancy," *International Journal of Cancer*, vol. 80, no. 3, pp. 356–364, 1999.

[15] J. Minarovits, L. F. Hu, S. Imai et al., "Clonality, expression and methylation patterns of the Epstein-Barr virus genomes in lethal midline granulomas classified as peripheral angiocentric T cell lymphomas," *Journal of General Virology*, vol. 75, no. 1, pp. 77–84, 1994.

[16] A. Jaccard, N. Gachard, B. Marin et al., "Efficacy of L-asparaginase with methotrexate and dexamethasone (AspaMetDex regimen) in patients with refractory or relapsing extranodal NK/T-cell lymphoma, a phase 2 study," *Blood*, vol. 117, no. 6, pp. 1834–1839, 2011.

[17] J. R. Anderson, J. O. Armitage, and D. D. Weisenburger, "Epidemiology of the non-Hodgkin's lymphomas: distributions of the major subtypes differ by geographic locations," *Annals of Oncology*, vol. 9, no. 7, pp. 717–720, 1998.

[18] H. Kohrt and R. Advani, "Extranodal natural killer/T-cell lymphoma: current concepts in biology and treatment," *Leukemia and Lymphoma*, vol. 50, no. 11, pp. 1773–1784, 2009.

[19] J. M. Vose, M. Neumann, and M. E. Harris, "International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes international T-cell lymphoma project," *Journal of Clinical Oncology*, vol. 26, no. 25, pp. 4124–4130, 2008.

[20] C. Bossard, K. Belhadj, F. Reyes et al., "Expression of the granzyme B inhibitor PI9 predicts outcome in nasal NK/T-cell lymphoma: results of a Western series of 48 patients treated with first-line polychemotherapy within the Groupe d'Etude des Lymphomes de l'Adulte (GELA) trials," *Blood*, vol. 109, no. 5, pp. 2183–2189, 2007.

[21] T. M. Kim, S. Y. Lee, Y. K. Jeon et al., "Clinical heterogeneity of extranodal NK/T-cell lymphoma, nasal type: a national survey of the Korean Cancer Study Group," *Annals of Oncology*, vol. 19, no. 8, pp. 1477–1484, 2008.

[22] W. Yong, W. Zheng, J. Zhu et al., "L-asparaginase in the treatment of refractory and relapsed extranodal NK/ T-cell lymphoma, nasal type," *Annals of Hematology*, vol. 88, no. 7, pp. 647–652, 2009.

[23] V. E. Reyes Jr., T. Al-Saleem, V. G. Robu, and M. R. Smith, "Extranodal NK/T-cell lymphoma nasal type: efficacy of pegaspargase. Report of two patients from the United Sates and review of literature," *Leukemia Research*, vol. 34, no. 1, pp. e50–e54, 2010.

[24] Y. Huang, A. De Reyniès, L. De Leval et al., "Gene expression profiling identifies emerging oncogenic pathways operating in extranodal NK/T-cell lymphoma, nasal type," *Blood*, vol. 115, no. 6, pp. 1226–1237, 2010.

[25] J. Iqbal, D. D. Weisenburger, A. Chowdhury et al., "Natural killer cell lymphoma shares strikingly similar molecular features with a group of non-hepatosplenic $\gamma\delta$ T-cell lymphoma and is highly sensitive to a novel aurora kinase A inhibitor in vitro," *Leukemia*, vol. 25, no. 2, pp. 348–358, 2011.

[26] T. Nagato, H. Kobayashi, K. Kishibe et al., "Expression of interleukin-9 in nasal natural killer/T-cell lymphoma cell lines and patients," *Clinical Cancer Research*, vol. 11, no. 23, pp. 8250–8257, 2005.

[27] Y. Nakashima, H. Tagawa, R. Suzuki et al., "Genome-wide array-based comparative genomic hybridization of natural killer cell lymphoma/leukemia: different genomic alteration patterns of aggressive NK-cell leukemia and extranodal NK/T-cell lymphoma, nasal type," *Genes Chromosomes and Cancer*, vol. 44, no. 3, pp. 247–255, 2005.

[28] S.-B. Ng, V. Selvarajan, G. Huang et al., "Activated oncogenic pathways and therapeutic targets in extranodal nasal-type NK/T cell lymphoma revealed by gene expression profiling," *Journal of Pathology*, vol. 223, no. 4, pp. 496–510, 2011.

[29] T. Oka, T. Yoshino, K. Hayashi et al., "Reduction of hematopoietic cell-specific tyrosine phosphatase SHP-1 gene expression in natural killer cell lymphoma and various types of lymphomas/leukemias: combination analysis with cDNA expression array and tissue microarray," *American Journal of Pathology*, vol. 159, no. 4, pp. 1495–1505, 2001.

[30] Y. Zhang, J. H. Ohyashiki, T. Takaku, N. Shimizu, and K. Ohyashiki, "Transcriptional profiling of Epstein-Barr virus (EBV) genes and host cellular genes in nasal NK/T-cell lymphoma and chronic active EBV infection," *British Journal of Cancer*, vol. 94, no. 4, pp. 599–608, 2006.

[31] H. E. Heslop, K. S. Slobod, M. A. Pule et al., "Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients," *Blood*, vol. 115, no. 5, pp. 925–935, 2010.

[32] A. K. S. Chiang, Q. Tao, G. Srivastava, and F. C. S. Ho, "Nasal NK- and T-cell lymphomas share the same type of Epstein-Barr virus latency as nasopharyngeal carcinoma and Hodgkin's disease," *International Journal of Cancer*, vol. 68, no. 3, pp. 285–290, 1996.

[33] C. M. Bollard, S. Gottschalk, A. M. Leen et al., "Complete responses of relapsed lymphoma following genetic modification of tumor-antigen presenting cells and T-lymphocyte transfer," *Blood*, vol. 110, no. 8, pp. 2838–2845, 2007.

[34] C. P. Fox, T. A. Haigh, G. S. Taylor et al., "A novel latent membrane 2 transcript expressed in Epstein-Barr virus-positive NK- and T-cell lymphoproliferative disease encodes a target for cellular immunotherapy," *Blood*, vol. 116, no. 19, pp. 3695–3704, 2010.

[35] T. Kaneko, J. Fukuda, T. Yoshihara et al., "Nasal natural killer (NK) cell lymphoma: report of a case with activated NK cells containing Epstein-Barr virus and expressing CD21 antigen, and comparative studies of their phenotype and cytotoxicity with normal NK cells," *British Journal of Haematology*, vol. 91, no. 2, pp. 355–361, 1995.

[36] J. Tabiasco, A. Vercellone, F. Meggetto, D. Hudrisier, P. Brousset, and J. J. Fournié, "Acquisition of viral receptor by NK cells through immunological synapse," *Journal of Immunology*, vol. 170, no. 12, pp. 5993–5998, 2003.

[37] H. Kimura, Y. Hoshino, H. Kanegane et al., "Clinical and virologic characteristics of chronic active Epstein-Barr virus infection," *Blood*, vol. 98, no. 2, pp. 280–286, 2001.

[38] S. E. Straus, "The chronic mononucleosis syndrome," *Journal of Infectious Diseases*, vol. 157, no. 3, pp. 405–412, 1988.

[39] J. I. Cohen, H. Kimura, S. Nakamura, Y. H. Ko, and E. S. Jaffe, "Epstein-Barr virus-associated lymphoproliferative disease in non-immunocompromised hosts: a status report and summary of an international meeting, 8-9 September 2008," *Annals of Oncology*, vol. 20, no. 9, pp. 1472–1482, 2009.

[40] M. L. Boulland, V. Meignin, K. Leroy-Viard et al., "Human interleukin-10 expression in T/natural killer-cell lymphomas: association with anaplastic large cell lymphomas and nasal natural killer- cell lymphomas," *American Journal of Pathology*, vol. 153, no. 4, pp. 1229–1237, 1998.

[41] J. Teruya-Feldstein, E. S. Jaffe, P. R. Burd et al., "The role of Mig, the monokine induced by interferon-γ, and IP-10, the interferon-γ-inducible protein-10, in tissue necrosis and vascular damage associated with Epstein-Barr virus-positive lymphoproliferative disease," *Blood*, vol. 90, no. 10, pp. 4099–4105, 1997.

[42] P. Kanavaros, M. L. Boulland, B. Petit, B. Arnulf, and P. Gaulard, "Expression of cytotoxic proteins in peripheral T-cell and natural killer-cell (NK) lymphomas: association with extranodal site, NK or T$\gamma\delta$ phenotype, anaplastic morphology and CD30 expression," *Leukemia and Lymphoma*, vol. 38, no. 3-4, pp. 317–326, 2000.

[43] E. Vivier, E. Tomasello, M. Baratin, T. Walzer, and S. Ugolini, "Functions of natural killer cells," *Nature Immunology*, vol. 9, no. 5, pp. 503–510, 2008.

[44] C. Schmitt, B. Ghazi, and A. Bensussan, "NK cells and surveillance in humans," *Reproductive BioMedicine Online*, vol. 16, no. 2, pp. 192–201, 2008.

[45] P. Parham, "MHC class I molecules and KIRS in human history, health and survival," *Nature Reviews Immunology*, vol. 5, no. 3, pp. 201–214, 2005.

[46] Y. Suto, K. Maenaka, T. Yabe et al., "Chromosomal localization of the human natural killer cell class I receptor family genes to 19q13.4 by fluorescence in situ hybridization," *Genomics*, vol. 35, no. 1, pp. 270–272, 1996.

[47] S. I. Khakoo and M. Carrington, "KIR and disease: a model system or system of models?" *Immunological Reviews*, vol. 214, no. 1, pp. 186–201, 2006.

[48] W. Haedicke, F. C. S. Ho, A. Chott et al., "Expression of CD94/NKG2A and killer immunoglobulin-like receptors in NK cells and a subset of extranodal cytotoxic T-cell lymphomas," *Blood*, vol. 95, no. 11, pp. 3628–3630, 2000.

[49] C. W. Lin, W. H. Lee, C. L. Chang, J. Y. Yang, and S. M. Hsu, "Restricted killer cell immunoglobulin-like receptor repertoire without T-cell receptor γ rearrangement supports a true natural killer-cell lineage in a subset of sinonasal lymphomas," *American Journal of Pathology*, vol. 159, no. 5, pp. 1671–1679, 2001.

[50] R. Lundell, L. Hartung, S. Hill, S. L. Perkins, and D. W. Bahler, "T-cell large granular lymphocyte leukemias have multiple phenotypic abnormalities involving pan-T-cell antigens and receptors for MHC molecules," *American Journal of Clinical Pathology*, vol. 124, no. 6, pp. 937–946, 2005.

[51] A. Dalloul, L. Laroche, M. Bagot et al., "Interleukin-7 is a growth factor for Sezary lymphoma cells," *Journal of Clinical Investigation*, vol. 90, no. 3, pp. 1054–1060, 1992.

[52] N. Ortonne, S. Le Gouvello, H. Mansour et al., "CD158K/KIR3DL2 transcript detection in lesional skin of patients with erythroderma is a tool for the diagnosis of Sézary syndrome," *Journal of Investigative Dermatology*, vol. 128, no. 2, pp. 465–472, 2008.

[53] E. Poszepczynska-Guigné, V. Schiavon, M. D'Incan et al., "CD158k/KIR3DL2 is a new phenotypic marker of sezary cells: relevance for the diagnosis and follow-up of sezary syndrome," *Journal of Investigative Dermatology*, vol. 122, no. 3, pp. 820–823, 2004.

[54] F. Takei, K. L. McQueen, M. Maeda et al., "Ly49 and CD94/NKG2: developmentally regulated expression and evolution," *Immunological Reviews*, vol. 181, pp. 90–103, 2001.

[55] C. W. Lin, Y. H. Chen, Y. C. Chuang, T. Y. Liu, and S. M. Hsu, "CD94 transcripts imply a better prognosis in nasal-type extranodal NK/T-cell lymphoma," *Blood*, vol. 102, no. 7, pp. 2623–2631, 2003.

[56] L. A. Fernandez, B. Pope, C. Lee, and E. Zayed, "Aggressive natural killer cell leukemia in an adult with establishment of an NK cell line," *Blood*, vol. 67, no. 4, pp. 925–930, 1986.

[57] S. Koizumi, H. Seki, and T. Tachinami, "Malignant clonal expansion of large granular lymphocytes with a Leu-11+, Leu-7- surface phenotype: in vitro responsiveness of malignant cells to recombinant human interleukin 2," *Blood*, vol. 86, no. 5, pp. 1065–1073, 1986.

[58] C. J. Froelich, V. M. Dixit, and X. Yang, "Lymphocyte granule-mediated apoptosis: matters of viral mimicry and deadly proteases," *Immunology Today*, vol. 19, no. 1, pp. 30–36, 1998.

[59] M. B. Barrie, H. W. Stout, M. S. Abougergi, B. C. Miller, and D. L. Thiele, "Antiviral cytokines induce hepatic expression of the granzyme B inhibitors, proteinase inhibitor 9 and serine proteinase inhibitor 6," *Journal of Immunology*, vol. 172, no. 10, pp. 6453–6459, 2004.

[60] B. A. Bladergroen, C. J. L. M. Meijer, R. L. Ten Berge et al., "Expression of the granzyme B inhibitor, protease inhibitor 9, by tumor cells in patients with non-Hodgkin and Hodgkin lymphoma: a novel protective mechanism for tumor cells to circumvent the immune system?" *Blood*, vol. 99, no. 1, pp. 232–237, 2002.

[61] C. S. Ng, S. T. H. Lo, J. K. C. Chan, and W. C. Chan, "CD56+ putative natural killer cell lymphomas: production

of cytolytic effectors and related proteins mediating tumor cell apoptosis?" *Human Pathology*, vol. 28, no. 11, pp. 1276–1282, 1997.

[62] K. Aozasa, T. Takakuwa, T. Hongyo, and W. I. Yang, "Nasal NK/T-cell lymphoma: epidemiology and pathogenesis," *International Journal of Hematology*, vol. 87, no. 2, pp. 110–117, 2008.

[63] T. Takakuwa, Z. Dong, S. Nakatsuka et al., "Frequent mutations of Fas gene in nasal NK/T cell lymphoma," *Oncogene*, vol. 21, no. 30, pp. 4702–4705, 2002.

[64] M. Yamaguchi, K. Kita, H. Miwa et al., "Frequent expression of P-glycoprotein/MDR1 by nasal T-cell lymphoma cells," *Cancer*, vol. 76, no. 11, pp. 2351–2356, 1995.

[65] Y. H. Ko, H. J. Ree, W. S. Kim, W. H. Choi, W. S. Moon, and S. W. Kim, "Clinicopathologic and genotypic study of extranodal nasal-type natural killer/T-cell lymphoma and natural killer precursor lymphoma among Koreans," *Cancer*, vol. 89, no. 10, pp. 2106–2116, 2000.

[66] Y. H. Ko, K. E. Choi, J. H. Han, J. M. Kim, and H. J. Ree, "Comparative genomic hybridization study of nasal-type NK/T-cell lymphoma," *Communications in Clinical Cytometry*, vol. 46, no. 2, pp. 85–91, 2001.

[67] L. L. Siu, V. Chan, J. K. C. Chan, K. F. Wong, R. Liang, and Y. L. Kwong, "Consistent patterns of allelic loss in natural killer cell lymphoma," *American Journal of Pathology*, vol. 157, no. 6, pp. 1803–1809, 2000.

[68] L. L. Siu, K. F. Wong, J. K. C. Chan, and Y. L. Kwong, "Comparative genomic hybridization analysis of natural killer cell lymphoma/leukemia: recognition of consistent patterns of genetic alterations," *American Journal of Pathology*, vol. 155, no. 5, pp. 1419–1425, 1999.

[69] H. S. Sun, I. J. Su, Y. C. Lin, J. S. Chen, and S. Y. Fang, "A 2.6 Mb interval on chromosome 6q25.2-q25.3 is commonly deleted in human nasal natural killer/T-cell lymphoma," *British Journal of Haematology*, vol. 122, no. 4, pp. 590–599, 2003.

[70] J. Yoon and Y. H. Ko, "Deletion mapping of the long arm of chromosome 6 in peripheral T and NK cell lymphomas," *Leukemia and Lymphoma*, vol. 44, no. 12, pp. 2077–2082, 2003.

[71] J. Iqbal, C. Kucuk, R. J. deLeeuw et al., "Genomic analyses reveal global functional alterations that promote tumor growth and novel tumor suppressor genes in natural killer-cell malignancies," *Leukemia*, vol. 23, no. 6, pp. 1139–1151, 2009.

[72] A. Kuma, M. Hatano, M. Matsui et al., "The role of autophagy during the early neonatal starvation period," *Nature*, vol. 432, no. 7020, pp. 1032–1036, 2004.

[73] M. M. Hippert, P. S. O'Toole, and A. Thorburn, "Autophagy in cancer: good, bad, or both?" *Cancer Research*, vol. 66, no. 19, pp. 9349–9351, 2006.

[74] M. E. Ray, G. Wistow, Y. A. Su, P. S. Meltzer, and J. M. Trent, "AIM1, a novel non-lens member of the $\beta\gamma$-crystallin superfamily, is associated with the control of tumorigenicity in human malignant melanoma," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 7, pp. 3229–3234, 1997.

[75] G. A. Martins, L. Cimmino, M. Shapiro-Shelef et al., "Transcriptional repressor Blimp-1 regulates T cell homeostasis and function," *Nature Immunology*, vol. 7, no. 5, pp. 457–465, 2006.

[76] C. A. Turner Jr., D. H. Mack, and M. M. Davis, "Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells," *Cell*, vol. 77, no. 2, pp. 297–306, 1994.

[77] L. Pasqualucci, M. Compagno, J. Houldsworth et al., "Inactivation of the PRDM1/BLIMP1 gene in diffuse large B cell lymphoma," *Journal of Experimental Medicine*, vol. 203, no. 2, pp. 311–317, 2006.

[78] G. L. Semenza, "Hif-1 and human disease: one highly involved factor," *Genes and Development*, vol. 14, no. 16, pp. 1983–1991, 2000.

[79] P. J. Jost and J. Ruland, "Aberrant NF-κB signaling in lymphoma: mechanisms, consequences, and therapeutic implications," *Blood*, vol. 109, no. 7, pp. 2700–2707, 2007.

[80] J. E. Darnell Jr., I. M. Kerr, and G. R. Stark, "Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins," *Science*, vol. 264, no. 5164, pp. 1415–1421, 1994.

[81] Q. Zhang, P. N. Raghunath, L. Xue et al., "Multilevel dysregulation of STAT3 activation in anaplastic lymphoma kinase-positive T/null-cell lymphoma," *Journal of Immunology*, vol. 168, no. 1, pp. 466–474, 2002.

[82] P. Coppo, V. Gouilleux-Gruart, Y. Huang et al., "STAT3 transcription factor is constitutively activated and is oncogenic in nasal-type NK/T-cell lymphoma," *Leukemia*, vol. 23, no. 9, pp. 1667–1678, 2009.

[83] P. P. Piccaluga, C. Agostinelli, A. Califano et al., "Gene expression analysis of peripheral T cell lymphoma, unspecified, reveals distinct profiles and new potential therapeutic targets," *Journal of Clinical Investigation*, vol. 117, no. 3, pp. 823–834, 2007.

[84] T. Wang, G. Niu, M. Kortylewski et al., "Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells," *Nature Medicine*, vol. 10, no. 1, pp. 48–54, 2004.

[85] D. Rotin and S. Kumar, "Physiological functions of the HECT family of ubiquitin ligases," *Nature Reviews Molecular Cell Biology*, vol. 10, no. 6, pp. 398–409, 2009.

[86] R. Schmitz, M. L. Hansmann, V. Bohle et al., "TNFAIP3 (A20) is a tumor suppressor gene in Hodgkin lymphoma and primary mediastinal B cell lymphoma," *Journal of Experimental Medicine*, vol. 206, no. 5, pp. 981–989, 2009.

[87] L. Quintanilla-Martinez, M. Kremer, G. Keller et al., "p53 mutations in nasal natural killer/t-cell lymphoma from mexico: association with large cell morphology and advanced disease," *American Journal of Pathology*, vol. 159, no. 6, pp. 2095–2105, 2001.

[88] M. Li, D. Chen, A. Shiloh et al., "Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization," *Nature*, vol. 416, no. 6881, pp. 648–653, 2002.

[89] M. Kimura, Y. Matsuda, T. Eki et al., "Assignment of STK6 to human chromosome 20q13.2 → q13.1 and a pseudogene STK6P to 1q41 → q42," *Cytogenetics and Cell Genetics*, vol. 79, no. 3-4, pp. 201–203, 1997.

[90] L. Zhang, M. S. Anglesio, M. O'Sullivan et al., "The E3 ligase HACE1 is a critical chromosome 6q21 tumor suppressor involved in multiple cancers," *Nature Medicine*, vol. 13, no. 9, pp. 1060–1069, 2007.

[91] M. S. Anglesio, V. Evdokimova, N. Melnyk et al., "Differential expression of a novel ankyrin containing E3 ubiquitin-protein ligase, Hace1, in sporadic Wilms' tumor versus normal kidney," *Human Molecular Genetics*, vol. 13, no. 18, pp. 2061–2074, 2004.

[92] J. Zhao, Z. Zhang, Z. Vucetic, K. J. Soprano, and D. R. Soprano, "HACE1: a novel repressor of RAR transcriptional activity," *Journal of Cellular Biochemistry*, vol. 107, no. 3, pp. 482–493, 2009.

[93] T. Bowman, R. Garcia, J. Turkson, and R. Jove, "STATs in oncogenesis," *Oncogene*, vol. 19, no. 21, pp. 2474–2488, 2000.
[94] J. F. Bromberg, M. H. Wrzeszczynska, G. Devgan et al., "Stat3 as an oncogene," *Cell*, vol. 98, no. 3, pp. 295–303, 1999.
[95] R. Chiarle, W. J. Simmons, H. Cai et al., "Stat3 is required for ALK-mediated lymphomagenesis and provides a possible therapeutic target," *Nature Medicine*, vol. 11, no. 6, pp. 623–629, 2005.
[96] A. Zamo, R. Chiarle, R. Piva et al., "Anaplastic lymphoma kinase (ALK) activates Stat3 and protects hematopoietic cells from cell death," *Oncogene*, vol. 21, no. 7, pp. 1038–1047, 2002.
[97] H. Yu, M. Kortylewski, and D. Pardoll, "Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment," *Nature Reviews Immunology*, vol. 7, no. 1, pp. 41–51, 2007.
[98] M. Kortylewski, M. Kujawski, T. Wang et al., "Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity," *Nature Medicine*, vol. 11, no. 12, pp. 1314–1321, 2005.
[99] C. Zou, J. Ma, X. Wang et al., "Lack of Fas antagonism by Met in human fatty liver disease," *Nature Medicine*, vol. 13, no. 9, pp. 1078–1085, 2007.
[100] B. K. Hadland, N. R. Manley, D. M. Su et al., "γ-secretase inhibitors repress thymocyte development," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 13, pp. 7487–7491, 2001.
[101] T. Palomero, M. L. Sulis, M. Cortina et al., "Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia," *Nature Medicine*, vol. 13, no. 10, pp. 1203–1210, 2007.
[102] E. Chanudet, H. Ye, J. Ferry et al., "A20 deletion is associated with copy number gain at the TNFAIBIC locus and occurs preferentially in translocation-negative MALT lymphoma of the ocular adnexa and salivary glands," *Journal of Pathology*, vol. 217, no. 3, pp. 420–430, 2009.
[103] L. L. Siu, J. K. C. Chan, K. F. Wong, and Y. L. Kwong, "Specific patterns of gene methylation in natural killer cell lymphomas: p73 is consistently involved," *American Journal of Pathology*, vol. 160, no. 1, pp. 59–66, 2002.
[104] V. Rouget-Quermalet, J. Giustiniani, A. Marie-Cardine et al., "Protocadherin 15 (PCDH15): a new secreted isoform and a potential marker for NK/T cell lymphomas," *Oncogene*, vol. 25, no. 19, pp. 2807–2811, 2006.
[105] M. Ito, T. Maruyama, N. Saito, S. Koganei, K. Yamamoto, and N. Matsumoto, "Killer cell lectin-like receptor G1 binds three members of the classical cadherin family to inhibit NK cell cytotoxicity," *Journal of Experimental Medicine*, vol. 203, no. 2, pp. 289–295, 2006.
[106] J. R. Bischoff and G. D. Plowman, "The Aurora/Ipl1p kinase family: regulators of chromosome segregation and cytokinesis," *Trends in Cell Biology*, vol. 9, no. 11, pp. 454–459, 1999.
[107] H. Katayama, K. Sasai, H. Kawai et al., "Phosphorylation by aurora kinase A induces Mdm2-mediated destabilization and inhibition of p53," *Nature Genetics*, vol. 36, no. 1, pp. 55–62, 2004.
[108] H. G. Drexler and Y. Matsuo, "Malignant hematopoietic cell lines: in vitro models for the study of natural killer cell leukemia-lymphoma," *Leukemia*, vol. 14, no. 5, pp. 777–782, 2000.
[109] S. Zhao, Q. L. Tang, M. X. He et al., "A novel nude mice model of human extranodal nasal type NK/T-cell lymphoma," *Leukemia*, vol. 22, no. 1, pp. 170–178, 2008.

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Review Article

Potential Role of NK Cells in the Pathogenesis of Inflammatory Bowel Disease

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NK cells are a major component of the innate immune system and play an important role in the tissue inflammation associated with autoimmune diseases such as inflammatory bowel disease (IBD). NK cells are unique in bearing both stimulatory and inhibitory receptors specific for MHC class I molecules, and their function is regulated by a series of inhibiting or activating signals. The delicate balance between activation and inhibition that decides NK cell final action provides an opportunity for their possible modulatory effect on specific therapeutic settings. Intestinal NK cells are phenotypically distinct from their counterparts in the blood and resemble "helper" NK cells, which have potentially important functions both in promoting antipathogen responses and in the maintenance of intestinal epithelial homeostasis. NK cell activities have been found to be significantly below normal levels in both remissive and active stages of IBD patients. However, some proinflammatory cytokines (e.g., IL-15, IL-21, and IL-23) could potently induce NK cell activation to secret high levels of proinflammatory cytokines (e.g., IFN-γ and TNF) and promote the cytolytic activities against the target cells. This paper provides the characteristics of intestinal NK cells and their potential role in the pathogenesis of IBD.

1. Introduction

Inflammatory bowel diseases (IBDs), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory diseases of the gastrointestinal tract. The two conditions share a number of common characteristics. However, notable differences are also observed in these disorders. CD may be patchy, segmental, and typically transmural inflammation in the gut, which is characterized by the aggregation of macrophages that frequently form noncaseating granulomas. On the contrary, UC shows the pathological features of a significant number of leukocyte infiltrations within the lamina propria and the crypts, where they form microabscesses, as well as depletion of mucin by goblet cells [1]. Although their exact etiology is still not completely understood, increasing data have demonstrated that these conditions occur through an inappropriate immune response to a subset of commensal enteric bacteria in a genetically susceptible host, with disease initiated by environmental triggers. Dysfunction of the mucosal immune system evokes intestinal inflammation through the activation of both innate and acquired immunity in the gut. Evidence has demonstrated that both T helper 1 (Th1) and Th2 cells are involved in the induction of chronic gut inflammation [2, 3]. CD is a predominately Th1- and Th17-mediated process, while UC seems to be a Th2-like disorder. In healthy individuals, such immunopathogenesis is avoided by the presence of regulatory T cells that inhibit the inflammatory pathway [4]. Among the innate immune compartments, NK cells are a major component of the innate immune responses against intracellular pathogens and participate in the tissue inflammation associated with autoimmune diseases, including IBD [5–7].

2. Immune Responses of NK Cells

NK cells are important effector cells of the innate immune system required for the first line of defense against transformed and infected cells and play an essential role in linking innate and adaptive immunity through their ability to secrete IFN-γ [5–7]. At the early stage of infection, NK cells are considered as the primary source of IFN-γ, shaping

the adaptive immunity through differentiation of $CD4^+$ T cells to the Th1 subsets [8, 9]. NK cells kill their target cells through two major pathways, both requiring close contact between NK cells and the target cells. In the first pathway, cytoplasmic granule toxins including perforins and granzymes are secreted by exocytosis and together induce apoptosis of the target cells. The second pathway involves the engagement of death receptors in target cells by their cognate ligands in NK cells, resulting in classical caspase-dependent apoptosis [8].

Previous studies [7] both in mouse models of autoimmune diseases and in humans have shown that NK cells have either a disease-promoting or -controlling role. Unlike T cells, NK cells do not express a diverse set of antigen-specific receptors, but they are unique in bearing both stimulatory and inhibitory receptors, and their function is regulated by a series of inhibiting or activating signals. When NK cell inhibitory receptors bind to major histocompatibility complex (MHC) class I molecules, their effector functions (i.e., cytotoxicity and cytokine production) are then blocked. Lower expression of stimulatory receptors could result from specific downregulation of the receptors in such NK cells, or from a failure of these cells to upregulate such receptors during development. Moreover, the activation of NK cells also results from the concerted action of costimulatory molecules already well characterized for their function in T cells. However, evidence indicates that NK cells also regulate the innate and acquired immune responses through their secretion of soluble factors and/or cell-cell contact [8]. NK cells discriminate from myeloid immature dendritic cells, which typically underexpress MHC class I molecules, and mature dendritic cells, which upregulate MHC class I expression after antigen uptake [10]. The killing of immature dendritic cells by NK cells has been interpreted as a control of the quality of dendritic cells, allowing only mature dendritic cells to migrate to the lymph nodes [11].

NK cells develop primarily in the bone marrow in adults and are widely distributed in the body, but the largest population can be found in spleen, lung, liver, bone marrow, and peripheral blood. NK cells can migrate to various tissues [12]. Intestinal NK cells are phenotypically distinct from their counterparts in the blood and resemble "helper" NK cells, which have potentially important functions both in promoting antipathogen responses and in the maintenance of intestinal epithelium [13]. It has been suggested that NK cells in gut, like T cells, require priming for activation, a process that involves cytokines such as IFN-γ, IL-15, and IL-18 [13].

3. Pathogenic Role of NK Cells in IBD

3.1. Alteration of Lamina Propria NK Cells in IBD. NK cells are present within the gut-associated lymphoid tissue including intraepithelial lymphoid compartment, intestinal lamina propria, Peyer's patches, and mesenteric lymphoid nodes and display a proinflammatory cytokine profile (e.g., IFN-γ, TNF, IL-2, IL-17, and IL-22) in response to commensal enteric bacteria through the innate immune system and cytolytic activity, indicating that intestinal NK cells play an important role in mucosal innate immunity and tolerance [13–17]. These mucosal NK cells are distinct from conventional NK cells in the periphery, characterized by the expression of a transcription factor, RORC in human or RORγt in mice, CD127 (IL-7Rα) and NKp44 in humans or NKp46 in mice. CD56 serves as an important marker defining functionally distinct subsets of NK and NKT cells based on their ability in the periphery. In humans, $CD56^{bright}$ NK cells show potential for cytokine secretion, while $CD56^{dim}$ NK cells have elevated cytotoxicity associated with a mature differentiation state. Similar subsets of NK cells are also found in mice based on their expression of CD27. Mucosal $CD56^+$ NK cells express a mature phenotype and produce high amounts of IFN-γ and TNF. A relative reduction in $CD56^+$ NK cells may conversely have an impact on intestinal epithelial repair processes in the gastrointestinal tract [18, 19]. Recently, $NKp46^+CD56^+CD3^-$ NK cells have been found in gut lamina propria surrounding colonic crypts and do not express MHC class I-specific killer cell immunoglobulin receptor KIR2D. Phenotypic analysis reveals that lamina propria $NKp46^+CD3^-$ NK cells are $CD127^+$c-$kit^+CD122^{low}CD27^{low}Ly49^-$. Importantly, these subsets of NK cells express RORγt, produce high level of IL-22 but not IFN-γ and IL-17 after stimulation *in vitro*, and lack NK cell cytolytic function [20–22]. These data indicate that $NKp46^+CD3^-$ NK cells in gut mucosa are distinct from conventional NK cells characteristic of IFN-γ production and cytotoxicity and may be involved in intestinal epithelial homeostasis and prevention of intestinal inflammation.

The precise role of NK cells in the pathogenesis of IBD is still elusive. Increasing evidence has indicated that distinct functional subsets of intestinal mucosal NK cells with an alteration in activation and cytotoxic activity potentially contribute to the pathogenesis of IBD [19, 23, 24]. NK cells have been found to be increased in inflamed mucosa of IBD patients, and NK cell differentiation is also accelerated in the lamina propria, suggesting that NK cells are involved in the disease pathophysiology. $CD16^+$ NK cells are found to be increased in the lamina propria from both CD and UC patients compared with healthy controls, and azathioprine preferentially inhibits proliferation of $CD16^+$ NK cells and induces apoptosis in resting but not in preactivated NK cells [23], indicating that NK cells with cytolytic potential are enriched in the colonic lamina propria of IBD patients and that azathioprine is associated with a reduction in these cells and a normalization of NK cell population in gut mucosa. However, previous work has reported that the populations of $CD161^+$ NK cells are significantly decreased in the inflamed mucosa of UC, whereas the frequency of conventional $CD161^+$ cells is similar among IBD patient and healthy controls. These data indicate that colonic lamina propria $CD161^+$ NK cells are thought to play important roles as anti-inflammatory cells and that the decrease in the proportions of these cells in inflamed colon may be associated with colonic inflammation progresses in IBD [24].

Although $NKp44^+$ or $NKp46^+$ IL-22-producing NK cells are present in intestinal mucosa, their role in the

pathogenesis of IBD is still unknown. Recent work has demonstrated that peripheral blood $CD56^+CD3^-$ NK cells strongly express NKp30, NKp46, CD122, NKG2D, and CD244, but not NKp44, CD127, and CD69 [25]. On the contrary, gut lamina propria $CD56^+CD3^-$ NK cells could express NKp30, NKp44, NKp46, CD122, CD127, NKG2D, CD244 and CD69. Interestingly, both $NKp44^+$ and $NKp46^+$ NK cells are found to be present in intestinal mucosa. $NKp44^-NKp46^+$ NK cells preferentially express high levels of CD122 but low levels of RORC and CD127 and produce IFN-γ after stimulation *in vitro*, whereas $NKp44^+NKp46^-$NK cells express high levels of RORC and CD127 and produce IL-22 [25]. These IFN-γ-producing $NKp46^+$ NK cells are found to be significantly increased in inflamed mucosa of CD patients, while IL-22-producing $NKp44^+$ NK cells are markedly decreased compared with those in UC patients and healthy controls. IL-23 could promote $NKp46^+$ NK cell activation to produce large amounts of IFN-γ. These data indicate that the balance between IFN-γ-producing $NKp46^+$ and IL-22-producing $NKp44^+$ NK cells is disrupted in inflamed mucosa of CD patients and that these $NKp46^+$ NK cells may be involved in the development of IBD.

3.2. NK KIR Genotypic Association with IBD. As part of the innate immune system, NK cells recognize human leukocyte antigen (HLA) class I molecules in target cells through their membrane receptors. The main receptors of NK cells are the killer immunoglobulin-like receptors (KIRs) [26]. The human KIR gene family comprises 15 genes and 2 pseudogenes, which are located within the IBD6 linkage region at chromosome 19q13.4. KIRs contain either two or three immunoglobulin-like domains with either long (2DL, 3DL) or short (2DS, 3DS) cytoplasmic tails. The presence of a long cytoplasmic tail (L) with immune tyrosine-based motifs (ITIM) permits the transduction of inhibitory signals and characterizes the inhibitory KIRs (2DL, 3DL), which inhibit NK- and cytotoxic T cell-mediated lysis of target cells expressed appropriate HLA class I ligands. In contrast, the presence of a short cytoplasmic tail (S) is associated with the activating or noninhibitory KIR (2DS, 3DS), which may promote cytolysis of target cells [26]. Therefore, a variety of inhibitory and activating KIRs which recognize and bind to their HLA class I ligands in target cells could regulate activation and inhibition of NK cell responses. Various KIR/HLA combinations may program the differentiation of NK cells during immune responses.

Recent genetic association studies have implicated that both KIR and their ligands display considerable genetic diversity in the development of several inflammatory conditions, including human IBD [26]. KIR and HLA C locus (HLA-Cw) variants that reduce NK cell inhibition have been shown to increase susceptibility to the development of IBD [27]. We have found that the KIR2DL1 and KIR2DL3 gene frequencies are significantly lower in UC patients compared with healthy controls (0.71 versus 0.896; 0.62 versus 0.821). The KIR2DL1 gene phenotype frequency is markedly decreased in CD patients more than healthy controls (0.731 versus 0.896). Interestingly, KIR2DL1–HLA-C2 combination is observed to be decreased in IBD patients compared with controls (0.38 versus 0.575 in UC; 0.404 versus 0.575 in CD) [27]. These data suggest that the decrease of combination KIR2DL1 and HLA-C2 may reduce the activating threshold of NK cells and CTL, enhance the cytolytic activity of lymphocytes, promote their multiplication, and finally lead to immune response to a subset of commensal enteric bacteria in IBD. Thus, KIR genotype and HLA ligand interaction may contribute to the genetic susceptibility of IBD.

3.3. Proinflammatory Cytokines in the Induction of NK Cell Activation in IBD. Previous work has proven that both stromal cells and cytokine/growth factors play a critical role in the development of NK cell development. Some proinflammatory cytokines (e.g., IL-2, IL-15, IL-21, and IL-23) have been observed to be involved in the immune responses of NK cells under inflammatory conditions such as IBD.

3.3.1. IL-2. IL-2 is a lymphocytotrophic cytokine that is involved in the growth and differentiation of T and B cells and enhances the cytolytic activities of NK cells. It induces the proliferation of $CD4^+$ and $CD8^+$ T cells by upregulating and maintaining the expression of the IL-2 receptor (IL-2R) α-subunit, which forms, together with the β-subunit and γ-subunit, the high-affinity IL-2R. IL-2 is also required for maturation and development of NK cells [28]. It has been documented that *in vitro* expanded NK cells have increased natural cytotoxicity receptors, TRAIL and NKG2D expression, and superior tumor cytotoxicity compared with short-term IL-2-activated NK cells. There is controversial about the amount of IL-2 in the mucosa of IBD patients. Some studies have shown that mRNA levels of IL-2 are increased in inflamed mucosa of active CD, while in other studies, both mRNA and protein levels of IL-2 are reduced in both CD and UC [29, 30]. IL-2 homeostasis may lead to preferential depletion of regulatory T-cell subsets, which cause exacerbation of inflammation in the gut [31]. In contrast, it has been conceived that eruption of IBD is associated with disturbed homeostasis and dominance of effector cells including colitogenic T-cell clones [32]. Therefore, the disruption of IL-2 signaling may evolve as a deleterious mechanism in the context of autoimmunity, rather than an immunosuppressive strategy [31]. Therefore, IL-2 secreted by intestinal mucosal T cells in IBD may contribute to NK homeostasis and the development of IBD.

3.3.2. IL-15. IL-15 is mainly derived from nonlymphoid cells and shares many similarities to IL-2. It has been also found to be produced by intestinal macrophages and other cell types in response to luminal bacterial stimulation and plays an important role in growth and differentiation of immune cells within the intestinal mucosa, including T and B lymphocytes, NK cells, macrophages, and monocytes [33]. IL-15 exerts most of these effects by binding to a heterotrimeric complex composed of the IL-2Rβ chain, the IL-2R γc chain, and the IL-15Rα chain. IL-15 induces T-cell proliferation and cytokine production, stimulates locomotion and chemotaxis

of normal T cells, and protects them from apoptosis. Importantly, IL-15 enhances NK cell cytotoxicity and antibody-dependent cell-mediated cytotoxicity and upregulates NK cell survival and production of NK cell-derived cytokines such as IFN-γ, GM-CSF, and TNF [28, 33]. Consistent with this, IL-15-deficient mice display a marked reduction of $CD8^+$ T cells, as well as certain intraepithelial lymphocytes. Incidentally, these mice also lack NK cells, suggesting that IL-15 may also be involved in expansion and survival of NK cells [34]. In rodent models of intracellular bacterial infections, evidence has demonstrated that IL-15 could attract NK cells to infected sites and limit bacterial colonization [35]. In the intestine IL-15 is observed to stimulate NK, NKT, and TCR$\gamma\delta$ T-cell activation [36]. However, after IL-15 treatment, both intraepithelial lymphocytes and NK cells have a greater killing potential against target cells [37]. Given that IL-15 is markedly increased in inflamed mucosa of IBD [38], IL-15 is also thought to be involved in the induction of NK cell activation and immune responses in IBD.

3.3.3. IL-21. IL-21 is a member of the IL-2 family of cytokines, expressed mainly by $CD4^+$ T cells, including Th1, Th2, and Th17 cells, and recently implicated in Th17 cell differentiation. IL-21R is structurally related to IL-2R and IL-15R and expressed in T, NK, B, and dendritic cells. IL-21 has been found to stimulate T-cell proliferation and differentiation, enhance clonal expansion of antigen-activated naïve $CD4^+$ and $CD8^+$ T cells, and induce the gene encoding IFN-γ, IL-18R, IL-2Rα, IL-12Rβ2, and the Th1-associated transcription factor T-bet in activated memory T cells [39, 40]. IL-21 also synergized with IL-15 and IL-18 in stimulating IFN-γ gene expression in these same cultures. Wurster et al. [41] reported that exposing naïve $CD4^+$ T cells to IL-21 under conditions that skew differentiation towards the Th1 phenotype actually inhibited IFN-γ production although it had little effect on other Th1 cytokines such as TNF or IL-2. IL-21 is also associated with the Th2-mediated immune response and plays a role in inhibiting the differentiation of naive Th cells into IFN-γ-producing Th1 cells. Moreover, IL-21 is found to promote human NK cell maturation and activation in synergy with IL-15, Ftl-3 ligand, and stem cell factor and enhance IFN-γ production and cytotoxicity [42].

IL-21 is involved in both cell-mediated and humoral responses and plays an important role in the pathogenesis of several autoimmune diseases, including IBD [39, 40]. Increased expression of IL-21 and IL-21R has been observed in the inflamed mucosa of IBD patients [43, 44]. We found that IL21R-positive cells are mainly expressed in $CD4^+$, $CD8^+$ T, B, and NK cells from peripheral blood and lamina propria of IBD patients [44]. IL-21 could expand already polarized IL-17A-producing cells in inflamed mucosa, promote a local inflammatory response in gut mucosa, and trigger intestinal mucosal T-cell activation and proinflammatory cytokine secretion [44]. Importantly, IL-21 could promote IBD NK cell activation to produce high levels of proinflammatory cytokines (e.g., IFN-γ and TNF) and enhance cytotoxicity against target cells. Our findings have confirmed that IL-21 is involved in the pathogenesis of IBD via the induction of NK cell activation and its cytolytic activity against target cells (e.g., intestinal epithelial cells). These data indicate that target immune therapy directed against IL-21R signaling may be warranted in some experimental colitis models, and that blockade of the IL-21R signaling pathway may have a therapeutic potential in IBD [45].

3.3.4. IL-23. IL-23 and IL-12 are members of a small family of proinflammatory heterodimer cytokines, sharing a common p40 subunit covalently linked either to a p35 subunit to form IL-12 or to a p19 subunit to form IL-23 [46]. IL-23R is predominantly expressed in T, NK, NKT cells, and, to a smaller extent, in monocytes, macrophages, and dendritic cells. After binding to the IL-23R, IL-23 preferentially induces memory T-cell activation. Evidence has demonstrated that IL-23 exhibits some similar biological activities to IL-12. However, in comparison with IL-12 with profound induction of Th1 immune response, as well as promotion of cytolytic, antimicrobial, and antitumor responses, IL-23 is found to play a critical role in the maintenance of immune response by controlling T-cell memory function and by influencing the proliferation and survival of IL-17-producing Th17 cells [47].

IL-23 has been reported to be increased expression in the sera and inflamed colon of IBD patients [48, 49]. Genome-wide association studies indicate that IL-23R is involved in the differentiation of Th17 cells, and is associated with susceptibility to CD and partly also to UC [50]. Our recent work has shown that the frequencies of IL-23R expression in $CD4^+$, $CD8^+$ T cells and NK cells are significantly increased in peripheral blood and lamina propria mononuclear cells from IBD patients compared with healthy controls [49]. Importantly, we found that IL-23 strongly induces IBD NK cell activation, showing increased secretion of proinflammatory cytokines (e.g., IFN-γ and TNF) and cytolytic activities. These findings indicate that IL-23 produced by intestinal mucosal macrophages/dendritic cells in inflamed mucosa of IBD could promote peripheral blood NK cell effector response. Recent work has demonstrated two distinct subsets of intestinal mucosal $NKp46^+$ NK cells according to the expression of RORγt. The RORγt^- subset functions as typical NK cells dependently on IL-15 but not on RORγt and displays NK cell activities (e.g., cytotoxicity and IFN-γ secretion), whereas the RORγt^+ subpopulation develops independently of IL-15 but required RORγt [21, 22]. Most interestingly, these $CD3^-NKp46^+$ cells located in the intestinal mucosa express RORγt and IL-22 but not IL-17A in response to IL-23 stimulation, and these cells lack normal NK cell function such as expression of perforin and IFN-γ, indicating that these NK cells play an important role in mucosal homeostasis and protectable immune response, particularly under microbial challenge [20]. These data suggest that intestinal mucosal NK cells may also be associated with proinflammatory response under inflammatory conditions and play an important role in mucosal homeostasis and defense against luminal microbial challenge although the precise role of IL-23 in the inductions

intestinal mucosal NK cell effect response need to be further investigated.

4. Conclusions

NK cells play an important role in linking innate and adaptive immunity. Evidence has proven that NK cells have a disease-promoting or -controlling role in autoimmune diseases, depending on the disease and the NK cell subset analyzed. Thus, ongoing studies should focus on intestinal mucosal NK cells and their interactions with other immune cells in inflamed mucosa of IBD, and these will provide powerful insights into potential role of NK cells in the pathogenesis of IBD.

Abbreviations

CD: Crohn's disease
HLA: Human leukocyte antigen
IBD: Inflammatory bowel disease
KIR: Killer immunoglobulin-like receptor
NK: Natural killer cells
UC: Ulcerative colitis.

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References

[1] R. J. Xavier and D. K. Podolsky, "Unravelling the pathogenesis of inflammatory bowel disease," *Nature*, vol. 448, no. 7152, pp. 427–434, 2007.

[2] R. B. Sartor, "Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis," *Nature Clinical Practice Gastroenterology and Hepatology*, vol. 3, no. 7, pp. 390–407, 2006.

[3] Z. J. Liu, P. K. Yadav, J. L. Su, J. S. Wang, and KE. Fei, "Potential role of Th17 cells in the pathogenesis of inflammatory bowel disease," *World Journal of Gastroenterology*, vol. 15, no. 46, pp. 5784–5788, 2009.

[4] T. T. Macdonald and G. Monteleone, "Immunity, inflammation, and allergy in the gut," *Science*, vol. 307, no. 5717, pp. 1920–1925, 2005.

[5] E. Vivier, E. Tomasello, M. Baratin, T. Walzer, and S. Ugolini, "Functions of natural killer cells," *Nature Immunology*, vol. 9, no. 5, pp. 503–510, 2008.

[6] A. H. Jonsson and W. M. Yokoyama, "Chapter 2 natural killer cell tolerance. Licensing and other mechanisms," *Advances in Immunology*, vol. 101, pp. 27–79, 2009.

[7] N. Schleinitz, E. Vély, J. R. Harlé, and E. Vivier, "Natural killer cells in human autoimmune diseases," *Immunology*, vol. 131, no. 4, pp. 451–458, 2010.

[8] A. Martín-Fontecha, L. L. Thomsen, S. Brett et al., "Induced recruitment of NK cells to lymph nodes provides IFN-γ for T(H)1 priming," *Nature Immunology*, vol. 5, no. 12, pp. 1260–1265, 2004.

[9] M. J. Smyth, E. Cretney, J. M. Kelly et al., "Activation of NK cell cytotoxicity," *Molecular Immunology*, vol. 42, no. 4, pp. 501–510, 2005.

[10] Y. Zhang, D. L. Wallace, C. M. de Lara et al., "*In vivo* kinetics of human natural killer cells: the effects of ageing and acute and chronic viral infection," *Immunology*, vol. 121, no. 2, pp. 258–265, 2007.

[11] A. Moretta, E. Marcenaro, S. Sivori, M. D. Chiesa, M. Vitale, and L. Moretta, "Early liaisons between cells of the innate immune system in inflamed peripheral tissues," *Trends in Immunology*, vol. 26, no. 12, pp. 668–675, 2005.

[12] T. Walzer, M. Bléry, J. Chaix et al., "Identification, activation, and selective in vivo ablation of mouse NK cells via NKp46," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 9, pp. 3384–3389, 2007.

[13] S. L. Sanos and A. Diefenbach, "Isolation of NK cells and NK-like cells from the intestinal lamina propria," *Methods in Molecular Biology*, vol. 612, pp. 505–517, 2010.

[14] F. Leòn, E. Roldán, L. Sanchez, C. Camarero, A. Bootello, and G. Roy, "Human small-iintestinal epithelium contains functional natural killer lymphocytes," *Gastroenterology*, vol. 125, no. 2, pp. 345–356, 2003.

[15] E. Vivier, H. Spits, and T. Cupedo, "Interleukin-22-producing innate immune cells: new players in mucosal immunity and tissue repair?" *Nature Reviews Immunology*, vol. 9, no. 4, pp. 229–234, 2009.

[16] H. Veiga-Fernandes, D. Kioussis, and M. Coles, "Natural killer receptors: the burden of a name," *Journal of Experimental Medicine*, vol. 207, no. 2, pp. 269–272, 2010.

[17] T. Cupedo, N. K. Crellin, N. Papazian et al., "Human fetal lymphoid tissue-inducer cells are interleukin 17-producing precursors to RORC CD127 natural killer-like cells," *Nature Immunology*, vol. 10, no. 1, pp. 66–74, 2009.

[18] O. Cohavy and S. R. Targan, "CD56 marks an effector T cell subset in the human intestine," *Journal of Immunology*, vol. 178, no. 9, pp. 5524–5532, 2007.

[19] S. C. Ng, S. Plamondon, H. O. Al-Hassi et al., "A novel population of human CD56 human leucocyte antigen D-related (HLA-DR+) colonic lamina propria cells is associated with inflammation in ulcerative colitis," *Clinical and Experimental Immunology*, vol. 158, no. 2, pp. 205–218, 2009.

[20] M. Cella, A. Fuchs, W. Vermi et al., "A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity," *Nature*, vol. 457, no. 7230, pp. 722–725, 2009.

[21] C. Luci, A. Reynders, I. I. Ivanov et al., "Influence of the transcription factor RORγt on the development of NKp46$^+$ cell populations in gut and skin," *Nature Immunology*, vol. 10, no. 1, pp. 75–82, 2009.

[22] S. L. Sanos, V. L. Bui, A. Mortha et al., "RORγt and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46$^+$ cells," *Nature Immunology*, vol. 10, no. 1, pp. 83–91, 2009.

[23] A. W. Steel, C. M. Mela, J. O. Lindsay, B. G. Gazzard, and M. R. Goodier, "Increased proportion of CD16(+) NK cells in the colonic lamina propria of inflammatory bowel disease patients, but not after azathioprine treatment," *Alimentary Pharmacology and Therapeutics*, vol. 33, no. 1, pp. 115–126, 2011.

[24] M. Shimamoto, Y. Ueno, S. Tanaka et al., "Selective decrease in colonic CD56(+) T and CD161(+) T cells in the inflamed mucosa of patients with ulcerative colitis," *World Journal of Gastroenterology*, vol. 13, no. 45, pp. 5995–6002, 2007.

[25] T. Takayama, N. Kamadax, H. Chinen et al., "Imbalance of NKp44(+)NKp46(-) and NKp44(-)NKp46(+) natural killer cells in the intestinal mucosa of patients with Crohn's disease," *Gastroenterology*, vol. 139, no. 3, pp. 882–892, 2010.

[26] S. I. Khakoo and M. Carrington, "KIR and disease: a model system or system of models?" *Immunological Reviews*, vol. 214, no. 1, pp. 186–201, 2006.

[27] H. Zhang, S. Liu, Z. Liu, and J. Li, "Expression of iKIR-HLA-Cw in patients with inflammatory bowel disease," *Life Science Journal*, vol. 5, no. 4, pp. 17–22, 2008.

[28] B. Becknell and M. A. Caligiuri, "Interleukin-2, interleukin-15, and their roles in human natural killer cells," *Advances in Immunology*, vol. 86, pp. 209–239, 2005.

[29] P. Desreumaux, E. Brandt, L. Gambiez et al., "Distinct cytokine patterns in early and chronic ileal lesions of Crohn's disease," *Gastroenterology*, vol. 113, no. 1, pp. 118–126, 1997.

[30] W. Hsu, W. Zhang, K. Tsuneyama et al., "Differential mechanisms in the pathogenesis of autoimmune cholangitis versus inflammatory bowel disease in interleukin-2R$\alpha^{-/-}$ mice," *Hepatology*, vol. 49, no. 1, pp. 133–140, 2009.

[31] K. Kameyamax, Y. Nemoto, T. Kanai et al., "IL-2 is positively involved in the development of colitogenic CD4+ IL-7Rα high memory T cells in chronic colitis," *European Journal of Immunology*, vol. 40, no. 9, pp. 2423–2436, 2010.

[32] E. C. Ebert, A. Panja, K. M. Das et al., "Patients with inflammatory bowel disease may have a transforming growth factor-β-, interleukin (IL)-2- or IL-10-deficient state induced by intrinsic neutralizing antibodies," *Clinical and Experimental Immunology*, vol. 155, no. 1, pp. 65–71, 2009.

[33] V. Budagian, E. Bulanova, R. Paus, and S. Bulfone-Paus, "IL-15/IL-15 receptor biology: a guided tour through an expanding universe," *Cytokine and Growth Factor Reviews*, vol. 17, no. 4, pp. 259–280, 2006.

[34] A. W. Goldrath, P. V. Sivakumar, M. Glaccum et al., "Cytokine requirements for acute and basal homeostatic proliferation of naïve and memory CD8$^+$ T cells," *Journal of Experimental Medicine*, vol. 195, no. 12, pp. 1515–1522, 2002.

[35] D. Jullien, P. A. Sieling, K. Uyemura, N. D. Mar, T. H. Rea, and R. L. Modlin, "IL-15, an immunomodulator of T cell responses in intracellular infection," *Journal of Immunology*, vol. 158, no. 2, pp. 800–806, 1997.

[36] N. Ohta, T. Hiroi, M. N. Kweon et al., "IL-15-dependent activation-induced cell death-resistant Th1 type CD8$\alpha\beta^+$NK1.1$^+$ T cells for the development of small intestinal inflammation," *Journal of Immunology*, vol. 169, no. 1, pp. 460–468, 2002.

[37] E. C. Ebert, "IL-15 converts human intestinal intraepithelial lymphocytes to CD94 produces of IFN-γ and IL-10, the latter promoting Fas ligand-mediated cytotoxicity," *Immunology*, vol. 115, no. 1, pp. 118–126, 2005.

[38] Z. Liu, K. Geboes, S. Colpaert, G. R. D'Haens, P. Rutgeerts, and J. L. Ceuppens, "IL-15 is highly expressed in inflammatory bowel disease and regulates local T cell-dependent cytokine production," *Journal of Immunology*, vol. 164, no. 7, pp. 3608–3615, 2000.

[39] R. Spolski and W. J. Leonard, "IL-21 is an immune activator that also mediates suppression via IL-10," *Critical Review of Immunology*, vol. 30, no. 6, pp. 559–570, 2010.

[40] R. Spolski and W. J. Leonard, "Interleukin-21: basic biology and implications for cancer and autoimmunity," *Annual Review of Immunology*, vol. 26, pp. 57–79, 2008.

[41] A. L. Wurster, V. L. Rodgers, A. R. Satoskar et al., "Interleukin 21 is a T helper (Th) cell 2 cytokine that specifically inhibits the differentiation of naive Th cells into interferon gamma-producing Th1 cells," *Journal of Experimental Medicine*, vol. 196, no. 7, pp. 969–977, 2002.

[42] J. Parrish-Novak, S. R. Dillon, A. Nelson et al., "Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function," *Nature*, vol. 408, no. 6808, pp. 57–63, 2000.

[43] G. Monteleone, I. Monteleone, D. Fina et al., "Interleukin-21 enhances T-helper cell type I signaling and interferon-γ production in Crohn's disease," *Gastroenterology*, vol. 128, no. 3, pp. 687–694, 2005.

[44] Z. Liu, L. Yang, Y. Cui et al., "IL-21 enhances NK cell activation and cytolytic activity and induces Th17 cell differentiation in inflammatory bowel disease," *Inflammatory Bowel Diseases*, vol. 15, no. 8, pp. 1133–1144, 2009.

[45] G. Monteleone, F. Pallone, and T. T. Macdonald, "Interleukin-21 as a new therapeutic target for immune-mediated diseases," *Trends in Pharmacological Sciences*, vol. 30, no. 8, pp. 441–447, 2009.

[46] B. Oppmann, R. Lesley, B. Blom et al., "Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12," *Immunity*, vol. 13, no. 5, pp. 715–725, 2000.

[47] T. Korn, E. Bettelli, M. Oukka, and V. K. Kuchroo, "IL-17 and Th17 cells," *Annual Review of Immunology*, vol. 27, pp. 485–517, 2009.

[48] C. Schmidt, T. Giese, B. Ludwig et al., "Expression of interleukin-12-related cytokine transcripts in inflammatory bowel disease: elevated interleukin-23p19 and interleukin-27p28 in Crohn's disease but not in ulcerative colitis," *Inflammatory Bowel Diseases*, vol. 11, no. 1, pp. 16–23, 2005.

[49] Z. Liu, P. K. Yadav, X. Xu et al., "The increased expression of IL-23 in inflammatory bowel disease promotes intraepithelial and lamina propria lymphocyte inflammatory responses and cytotoxicity," *Journal of Leukocyte Biology*, vol. 89, no. 4, pp. 597–606, 2011.

[50] R. H. Duerr, K. D. Taylor, S. R. Brant et al., "A genome-wide association study identifies IL23R as an inflammatory bowel disease gene," *Science*, vol. 314, no. 5804, pp. 1461–1463, 2006.

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Review Article

NK Cells and Psoriasis

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Psoriasis is a chronic condition of the skin characterised by distinctive scaly plaques. The immune system is now thought to play a major role in the development and pathogenesis of psoriasis with immune cells and cytokines influencing keratinocyte function. Keratinocytes in turn, can activate and recruit immune cells leading to a positive feedback loop in disease. Natural Killer (NK) cells are lymphocytes that are best known for killing virally infected and cancer cells. However, evidence is emerging to support a role for NK cells in psoriasis. NK cells are found in the inflammatory infiltrate in psoriatic skin lesions. They can produce a range of inflammatory cytokines, many of which are important in the pathogenesis of psoriasis. Recent genetic studies have identified a range of potential molecules relating to NK cell biology that are known to be important in psoriasis. This paper will discuss the evidence, both cellular and genetic, for NK cell involvement in psoriasis.

1. Psoriasis as an Inflammatory Disease

Psoriasis is chronic inflammatory condition of the skin with significant morbidity, affecting approximately 2% of the Caucasian population. The most common form of the disease, responsible for up to 90% of cases, is psoriasis vulgaris [1] and this paper will primarily deal with this form. It is characterised by demarcated, red, raised, scaly plaques that typically manifest on the elbows, knees, and scalp [1, 2]. Psoriasis guttate occurs in about 10% of patients [3] and displays small, scattered plaques [2, 4]. This form may develop into psoriasis vulgaris [4]. Pustular psoriasis is an uncommon form of the disease consisting of raised pus-filled bumps and large areas of reddened skin [4]. A proportion of psoriasis patients will develop psoriatic arthritis (PsA), a debilitating joint disease [2–4].

Psoriatic skin is marked by increased proliferation of keratinocytes, the major cell of the outermost layer of skin, resulting in a thickening of the epidermis. Altered differentiation and rapid maturation of keratinocytes is observed, as is parakeratosis, a process whereby keratinocytes retain their nuclei as they rise into the stratum corneum. The granular layer of the epidermis is reduced or absent and downward projections of the epidermis, known as rete, become elongated. There is marked angiogenesis and infiltration of immune cells into the skin [1, 2].

The cause of psoriasis is still unknown although it is clear that there is a strong genetic component to the disease. Several immune genes have been associated with psoriasis with the major histocompatibility complex on chromosome 6 being strongly implicated [5, 6]. Outbreaks of psoriasis can occur at sites of physical trauma and streptococcal infections have been particularly linked to psoriasis guttate, perhaps indicating a role for molecular mimicry [4]. There is some evidence that psoriasis may be an autoimmune disease; it shares many characteristics with multiple sclerosis and diabetes mellitus type 1 [7, 8], but as yet no autoantigens or self-reactive T-cells have been identified [6, 9].

There are a host of treatments for psoriasis ranging from topical creams to systematic drugs and phototherapy. Many effective treatments act on the immune system with TNF-α and T-cells being the common targets [2]. As our understanding of the disease immunopathogenesis expands, new therapeutic strategies targeting the immune system are being developed. Recent drugs targeting the IL-12/IL-23 family of cytokines has indicated this as a promising new treatment pathway for psoriasis [1, 10] and illuminates the effectiveness of targeting the immune system for treatment of this disease.

For much of its history psoriasis was believed to be solely a disorder of the skin characterised by aberrant keratinocyte activity. However, with increased understanding of the disease, a fundamental role for the immune system in its pathogenesis and maintenance has been established. Evidence for immune involvement in the course of psoriasis arose from several sources. The presence of a large number of immune cells in psoriatic skin suggested that they play a part in the disease and the discovery that therapies targeting the immune system were effective for the treatment of psoriasis further highlighted its importance. The curing of psoriasis following bone marrow transplantation from a healthy donor to a psoriatic host, and the inverse observation of the development of psoriasis after transplantation of bone marrow from a psoriatic donor to a healthy host both indicated the strong part played by the immune system in this disease [1].

Although the field has moved away from the idea of psoriasis being a disease only involving the skin, it is clear that resident skin cells do play a substantial role. Keratinocytes are believed to play an important part in the recruitment and activation of immune cells. Keratinocytes are themselves capable of producing proinflammatory cytokines such as IL-1, IL-6, and TNF-α, as well as antimicrobial peptides and chemokines that can stimulate immune cell migration to the skin [11, 12]. Keratinocytes are also highly responsive to cytokines secreted from immune cells. These may induce the development of psoriatic features [2], trigger the expression of adhesion molecules for immune cells or drive further production of inflammatory cytokines from keratinocytes [5], thus contributing to maintenance of the disease state. Vascular endothelial cells in psoriatic skin also possess adhesion molecules such as ICAM-1, VCAM-1, and E-selectin [2, 5]. These molecules are usually found in the lymph nodes and allow the adherence of immune cells. Immune cells resident in the skin may also play an important part in the disease with some authors suggesting the local immune response may be sufficient for development of lesions [13].

The role of the innate immune system in psoriasis is increasingly seen as important. Neutrophils are found in the stratum corneum of psoriatic skin [2]. As these cells are shortlived (approx 3 days), their sustained presence suggests that they are continually recruited. Dendritic cells (DCs) are increased in psoriatic lesions and are believed to contribute to shaping the T-cell response [1, 2]. DC subsets not usually found in the skin are also observed. Plasmacytoid DCs are potent producers of IFN-α, which is thought to be a key cytokine in triggering lesion development, and myeloid DCs, with the ability to secrete TNF-α and inducible nitric oxide synthase, have been also been observed in psoriatic skin [2, 5, 13]. There are increased numbers of mature and activated DCs in psoriatic lesions [5] implying that these cells may be stimulating other aspects of the immune response.

Natural killer T (NKTs) cells are a subset of lymphocytes that bear the T-cell receptor along with classical NK cell molecules including CD56, CD16, CD161, and CD94 [14, 15]. They have a limited T-cell receptor repertoire and are activated following recognition of glycolipid presented by the MHC class-1-like molecule CD1d [11, 14]. Interestingly, keratinocytes express CD1d and its expression is upregulated in psoriatic skin [16, 17]. It has been shown that NKT cells cultured with keratinocytes were activated to produce large amounts of IFN-γ [16, 18]. While additional studies are required to clarify possible changes in NKT populations in psoriasis, there is evidence of their increased expression within psoriatic lesions [14]. Previous data on circulating NKT cells in psoriasis is conflicting, with some authors observing no difference [7], while others finding a reduction in their numbers in patients relative to controls with this number increasing following treatment [15]. Evidence from animal models of psoriasis also implies a role for NKT cells with some studies reporting the ability of these cells to induce psoriasis in xenografted mice [17–19].

Much of the work investigating the immunopathogenesis of psoriasis focuses on T lymphocytes and their importance in the disease is widely accepted. Both $CD4^+$ and $CD8^+$ T-cells have been found in lesional skin with CD4 cells primarily in the dermis and CD8 cells in the epidermis [4, 5, 9]. While initially it was believed that psoriasis was mediated by IFN-γ-producing type 1 T-cells, it is now thought that the recently characterised Th17 subset also has a significant role [1, 6]. This subset is noted for the production of IL-17 and IL-22, two cytokines which are present in psoriatic lesions, in response to IL-23 [20, 21]. The expression of activation markers by both $CD4^+$ and $CD8^+$ cells has been observed and most of the T-cells in psoriatic lesions also express the memory cell antigen, CD45RO [5]. Interestingly, data from variable T-cell receptor genes indicates the presence of clonal T-cell populations within psoriatic lesions. This implies the selective expansion of T-cells bearing receptors of the same antigen specificity. However, as yet no antigen has been identified as the trigger for psoriasis. It is postulated that the observed clonality of T-cells may be due to the presence of unidentified pathogen antigens, of a bacterial superantigen, or of host autoantigens possibly coming into play due to molecular mimicry [4, 11].

2. A Role of NK Cells in Psoriasis?

NK cells are lymphocytes that are generally considered to be part of the innate immune system. They are best known for their ability to kill virally infected and cancer cells; however, they also produce a range of cytokines including IFN-γ, TNF-α, and TGF-β. They can be defined phenotypically by the presence of particular surface antigens: either NKp46 or $CD56^+CD3^-$ cells. While the role of NK cells in psoriasis still remains relatively unstudied, there is mounting evidence that these cells may contribute to disease. Ottaviani et al. [22] found that the inflammatory infiltrate into psoriatic skin consisted of 5–8% cells that expressed the NK cell phenotype of $CD56^+CD3^-$. Most of these were of the $CD56^{bright}$ subset of NK cells. Thought to represent more immature cells, $CD56^{bright}$ cells are less cytotoxic and more proficient at cytokine secretion compared to $CD56^{dim}$ NK cells. The cells present in the infiltrate found by Ottaviani et al. also expressed the activation antigen, CD69, and produced large quantities of IFN-γ *in vitro* in response to IL-2 stimulation. Supernatants from these IL2-stimulated NK cells

induced activation of keratinocytes causing upregulation of MHC class I molecules and induction of the expression of ICAM1 and HLA-DR receptors. The keratinocytes were also observed to secrete chemokines that are known to attract NK cells (CXCL10, CCL5, and CCL20) thereby providing a mechanism of NK cell recruitment to the skin. Indeed, receptors for these chemokines were identified on NK cells found in psoriatic skin, with high levels of CXCR3 and CCR5 (receptors for CXCL10 and CCL5, resp) and moderate levels of CCR6 (CCL20 receptor) expressed [22]. In this study, there were ten patients examined; thus, the results need validation in other cohorts. In addition, the study examined cells in lesions but did not compare the findings to either uninvolved skin or normal healthy skin. Therefore, it is unclear as to whether the data represent the phenotype expected in normal skin or if it represents an altered phenotype associated with psoriasis. The three chemokine receptors noted in this study, CXCR3, CCR6, and CCR5 have also been recently identified as mediators of NK cell recruitment to the skin in allergic contact dermatitis [23]. In terms of NK chemotaxis to psoriatic skin, evidence has recently emerged for a role for chemerin which is found in psoriatic lesions and is known to attract cells expressing the receptor CMKLR1 [24]. The $CD56^{dim}$ subset has been noted to express this receptor [25] and migration of these cells in response to chemerin has been shown [24]. The relevance of this finding regarding NK cells in psoriasis remains to be explored.

In another study, Cameron et al. found increased frequencies of cells expressing CD16 or CD57 antigens in psoriatic skin lesions compared to either uninvolved skin or skin from normal healthy individuals [7]. These changes were found in both epidermal and papillary dermal skin layers. Neither of these markers is ideal for detecting NK cells that are more accurately defined by either NKp46 or a combination of $CD56^{+}CD3^{-}$. The authors also looked at cells expressing the NK cell receptors, CD94, and 2DL1, and found the frequency of cells expressing these elevated in the papillary dermis but not in other skin layers [7]. In this study, samples numbers were low ($n = 10$ patients and $n = 4$ normal controls) and would thus benefit from further investigations.

In addition to directly studying receptors expressed by NK cells, changes in NK cell ligands in psoriasis can provide evidence supporting a role for NK cells in the disease as alterations in ligand expression can directly affect NK cell function (Figure 1). HLA-G, a nonclassical class I molecule, is expressed in psoriatic skin lesions but not in healthy skin [26, 27]. HLA-G is recognised by 2DL4, a member the killer cell immunoglobulin-like receptors (KIRs) expressed by all NK cells, and LILRB1, which belongs to a related family of immunoglobulin-like receptors and also expressed by human NK cells. The leader sequence of the HLA-G protein may also be presented by the HLA-E molecule allowing recognition by another set of receptors found on NK cells, the CD94/NKG2 dimers [28]. LILRB1 has been found on T-cells in psoriatic skin lesions [26]; however, whether NK cells receptors for HLA-G are modulated in psoriasis has yet to be determined.

Although psoriasis is a skin condition, changes in peripheral blood NK cells have been reported. A decrease in circulating NK cells was found in patients with chronic psoriasis with lower frequency of cells expressing common NK cell markers CD56, CD16, CD94 and 2DL1 (CD158a) present. The extent of the decrease in circulating NK cell numbers did not correlate to the clinical severity of disease [8]. However, this decrease in peripheral blood NK cell frequency was not observed in a recent study in patients with new-onset psoriasis, with levels of 2B4, CD48, NKG2D, CD16, and CD56 unchanged between patients and healthy controls [29] or in a study focusing on NK cell chemotaxis to chemerin where percentages of $CD56^{dim}CD16^{+}$ and of $CD56^{bright}CD16^{-}$ in the peripheral blood showed no difference between patients and controls [24]. Further to this, a study primarily investigating a role for NKT cells in psoriasis utilising multicolour CD56 and CD3 staining, reported no difference in the percentage of circulating NK cells in psoriasis compared to healthy controls [15]. Finally, preliminary data from our own lab also using multicolour staining to accurately define NK cells does not indicate a difference in NK cell numbers in the blood of patients compared to healthy controls (S. Dunphy, data not shown).

Phenotypic differences have been noted between NK cells in the blood of psoriasis patients compared to healthy controls. It was found that psoriatic NK cells had increased expression of the apoptosis-associated Fas receptor, and lower expression of CD94 and NKG2A. Cell surface expressions of 2B4, CD48, CD16, NKG2D, and CD56 were unchanged between patients and healthy controls [29]. Previous work has also indicated that Fas may have an important role in the pathogenesis of psoriasis. While it is best known for its ability to induce apoptosis following engagement of Fas ligand (FasL), this receptor is also able to cause the production of proinflammatory cytokines including TNF-α, a key cytokine in psoriasis. Further evidence for a role for Fas was provided by Gilhar et al. who showed that SCID mice that receive grafts of uninvolved psoriatic skin from patients combined with injection of IL2-stimulated NK cells developed characteristics of psoriasis, and that the blocking of the Fas/FasL interaction could prevent these changes [30].

In addition to cell surface receptors, molecules important in NK cell functions, for example, cytotoxicity, have been implicated in psoriasis although the data are conflicting. In one study, perforin, a pore-forming protein found in the cytotoxic granules of NK cells and a key mediator of cytotoxicity, was expressed at higher levels in the lesional psoriatic skin relative to uninvolved psoriatic or healthy control skin [31]. Psoriasis patients also had higher levels of perforin in their peripheral blood lymphocytes compared to healthy controls. However, the source of this perforin does not seem to be circulating NK cells, as no difference between the number of $CD56^{+}Perforin^{+}$ or $CD16^{+}Perforin^{+}$ cells was observed in psoriasis patients compared to controls [32]; it seems likely that CTLs are responsible for the elevated perforin levels. Another study has given evidence for a role of NK cell-produced perforin in psoriasis, with a significantly higher percentage of $CD56^{+}Perforin^{+}$ found in the peripheral blood of patients with severe disease versus

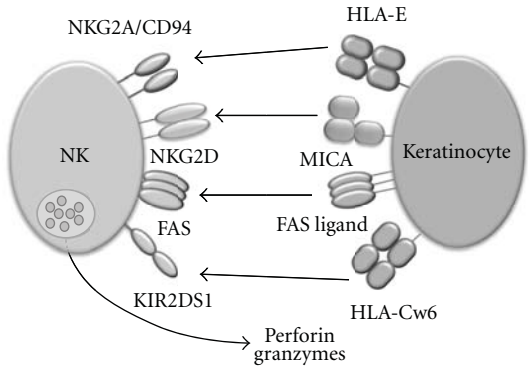

Figure 1: NK cells can interact with keratinocytes through a range of cell surface receptors. NK cells express cell surface receptors that regulate their interactions with other cell types including keratinocytes. Among these receptors is the NKG2A/CD94 inhibitory receptor that recognises and binds to HLA-E on target cells. NK cells also express a number of activating receptors including NKG2D which recognises MICA/B stress antigen and the Fas receptor which can activate cytokine secretion by NK cells. The activating KIR receptor 2DS1 (and its inhibitory counterpart, 2DL1) binds the HLA-Cw6 molecule and HLA-Cw6 is the strongest genetic association known in psoriasis. There is evidence in the literature to suggest that these receptors play a role in psoriasis. Activated NK cells are triggered to release their cytotoxic granule contents which contain perforin and granzymes.

those with mild psoriasis, but interestingly this difference was not observed comparing individuals with severe psoriasis to healthy controls. It was also noted that the vast majority of blood cells expressing CD16 were also positive for perforin in those with severe disease while a significantly lower frequency of CD16 positive cells coexpressing perforin was found in patients with mild psoriasis [33]. Cells expressing granzyme B, a serine protease that is released from NK cell granules and that triggers DNA degradation in target cells, have also been found in significantly higher numbers in involved psoriatic skin compared to uninvolved psoriatic and healthy skin [31].

3. NK-Cell-Associated Cytokines Play Important Roles in Psoriasis

Cytokines are small soluble proteins that are secreted from cells and influence many aspects of cell biology including proliferation, differentiation, and activation. They are key immunological regulators and altered cytokine profiles have been implicated in a host of diseases. Many studies have conclusively demonstrated that cytokines play an important role in the pathogenesis of psoriasis. Several of these cytokines are important in NK cell biology.

IL-12 is a key cytokine in the proliferation and activation of both NK and T-cells. It triggers the production of the proinflammatory cytokines TNF-α and IFN-γ from these cells and promotes the development of Th1 type effector T-cells [12]. IL-12 is a heterodimeric cytokine composed of a p35 subunit and a p40 subunit which come together to form a 70 kDa protein [34]. This p70 protein has been found in increased levels in psoriatic lesions. The p40 subunit has also been implicated in psoriasis but current evidence suggests that this may be due to its presence as part of IL-23, a related cytokine. No differences have yet been observed concerning the p35 subunit. There are conflicting reports regarding the levels of IL-12 in the serum of psoriasis patients, with some authors finding decreased levels while others report no change compared to healthy controls [12].

IL-23 is a proinflammatory cytokine with emerging links to autoimmunity [10]. It is a member of the IL-12 cytokine family and consists of a unique p19 subunit, and a p40 subunit that it shares with IL-12 [10, 34]. It is produced by activated macrophages and dendritic cells [34] and is involved in the development of Th17 cells [35]. The Th17 subset of $CD4^+$ T-cells have been identified as important cells in autoimmune diseases and IL-23 is thought to enhance secretion of both IL-22 and IL-17 from these cells [20]. IL-23 is also key in stimulating secretion of IL-22 from NK22 cells (discussed later in review). Memory T-cells are also stimulated to secrete IFN-γ by IL-23 [12, 36]. Genetic associations strongly support a role for IL-23 in psoriasis (see section below). In addition, IL-23 is highly expressed in lesional psoriatic skin and the expression of both subunits is significantly higher in lesions compared to normal skin [12]. It has been demonstrated that keratinocytes are capable of IL-23 production and that IL-23 expression is enhanced in keratinocytes of psoriatic patients [36]. Furthermore, therapies that target blocking of IL-23 have been shown to be effective in the treatment of psoriasis [10, 12].

Another key cytokine is IL-15 which is important for NK cell development, survival, and activation. It also acts to sustain inflammatory responses, promote angiogenesis, and suppress apoptosis [12]. It has been shown to trigger the production of IFN-γ, TNF-α, and IL-17 [37]. Keratinocytes have been shown to express IL-15 and its unique receptor subunit, the IL-15R α-chain, on their cell surface. It has been demonstrated that IL-15 is capable of inhibiting keratinocyte apoptosis; this may be relevant in psoriasis where abnormally low levels of apoptosis are displayed. IL-15 is highly expressed in psoriatic lesions compared to normal skin, as are binding sites for this cytokine [38]. Further evidence of a role for IL-15 in psoriasis is supplied by the observation that an antibody which interferes with IL-15R binding alleviated psoriasis in a mouse model of the disease [37].

Also important is IL-18 cytokine. It is produced in the immune response to pathogens and works together with IL-12 to drive IFN-γ production from NK and T-cells. It also stimulates the production of IL-13 and IL-4 from cells of the innate and adaptive immune response including NK cells, T-cells, basophils, and mast cells. IL-18 is thought to help drive inflammation in psoriasis. Lesional psoriatic skin contains higher levels of IL-18 mRNA compared to nonlesional or healthy skin, and increased expression of IL-18 is found in both involved and uninvolved psoriatic skin compared to healthy controls. Increased IL-18 has also been reported in plasma from psoriatic patients compared to healthy controls [12].

IL-22 is a member of the IL-10 family of cytokines [20, 39, 40]. It is produced by Th1 and Th17 cells [21, 39].

Recently, a novel population of NK cells, named NK22 cells, have been shown to secrete IL-22 [41]. IL-22 has a protective role at mucosal sites but has also been seen to be detrimental in autoimmune diseases by promoting inflammation [40]. The IL-22 receptor is expressed on keratinocytes [21, 39] and this cytokine has been shown to trigger keratinocyte production of several antimicrobial proteins, such as β-defensin 2, which have heightened levels in psoriasis [21]. Elevated levels of IL-22 mRNA have been reported in psoriatic lesions. Levels of IL-22 cytokine in the blood have been shown to be higher in psoriasis patients and cytokine level correlated with the disease severity [39]. *Ex vivo* treatment of keratinocytes with IL-22 results in the development of psoriatic features including hyperplasia, parakeratosis, downward epidermal projections, and acanthosis [21]. Importantly, IL-22 causes psoriasis-like skin changes in IL-22 transgenic mice; this effect was enhanced by TNF-α, a key cytokine in psoriasis [39].

IFN-γ is a cytokine that plays a large number of roles in the immune response. It is secreted by NK cells and T-cells [42] and stimulates the release of numerous proinflammatory cytokines from various cell types. It has roles in the activation, growth, and differentiation of lymphocytes and stimulates the cytotoxic activities of NK cells, CTLs, and macrophages. Studies on serum levels of IFN-γ in psoriasis have yielded conflicting results, with some authors noting no change relative to healthy controls. However, there have also been reports of highly elevated levels of IFN-γ in the serum and blister fluid of patients; levels correlated with disease severity and decreased after treatment. Studies investigating IFN-γ levels in the skin also vary with some reports observing stronger expression of IFN-γ in both involved and uninvolved psoriatic skin than in the skin of healthy controls [12].

TNF-α is produced by a variety of cells including NK cells [43]. TNF-α has amassed substantial interest in psoriasis where it appears to be a key mediator of the proinflammatory cascade. TNF-α appears to be locally produced in psoriatic lesions and its synthesis has been reported to be increased in involved psoriatic skin compared to uninvolved or healthy skin. Expression of the TNF receptor 1 is altered in lesional skin and soluble receptor is also found in higher levels in the blood of psoriatic patients. Several studies have found increased levels of TNF-α in the serum of psoriasis patients with a positive correlation to disease severity noted. Peripheral blood mononuclear cells isolated from patients showed increased capacity for TNF-α production *in vitro*. TNF-α is the most widely studied and utilized target for anticytokine psoriasis therapies and several antibodies that interfere with its functions including etanercept, infliximab and adalimumab are all highly effective in the treatment of psoriasis [12].

It is clear that many cytokines known to be important in psoriasis have strong links with NK biology and are either produced by NK cells (IFN-γ, TNF-α, and IL-22) or are important in their activation (IL-15, IL-18, IL-12, and IL-23). The nature of the relationship between these NK cell associated cytokines, NK cells, and psoriasis remains to be explored.

4. Recent Advances in Genetic Analysis of Psoriasis

Analysis of psoriasis among twins supports that there is a strong genetic basis for the development of psoriasis. In Northern Europeans, the rate of concordance is approximately 72% among monozygotic twins compared to approximately 15–23% of dizygotic twins. In other populations, the trend is similar although the percentages differ [44–46]. However, identification of the genetic loci involved has not been straightforward. It is clear that no one genetic locus causes psoriasis but rather several loci may contribute to the phenotype of psoriasis. Thus, psoriasis is a complex genetic disease. Early studies examined a role for MHC genes (HLA in human) and identified HLA-Cw6 as a locus associated with psoriasis [47]. There have been approximately ten genomewide linkage scans performed and over 20 possible regions linked to psoriasis identified; however, many of these associations identified have not been replicated in other studies [48]. More recently, several genomewide association scans (GWASs) have been performed and they have identified many genes important in the development and pathogenesis of psoriasis. A GWAS is a modern genetic tool that scans the entire genome at defined single nucleotide polymorphisms (SNPs). These are compared between control and disease groups, and genetic markers that have an altered frequency in disease can be identified. Sometimes the SNPs are located within known genes and thus a putative functional role for that gene in disease can be predicted. However, some of the SNPs reside outside genes and thus act as a marker for genes in that area. Identification of a SNP does not necessarily identify a gene of interest but rather it identifies an area of a chromosome that contains a gene that contributes to the phenotype.

Studies in both Asian and European populations have been performed and several loci have been confirmed as important in psoriasis. The most significant association has been with the MHC class I region of chromosome 6 that includes HLA-A, -B, -C, and HLA-E genes [6, 49]. Data suggest that the SNPs associated with psoriasis in this region are closest to HLA-C gene and further analysis at the allele level suggested that associated SNP alleles strongly correlate with HLA-Cw6, confirming the original candidate gene genetic studies [50]. There are three other genes in this HLA class I region that have well-defined roles in NK cell biology: HLA-B, HLA-E, and MICA. HLA-B genes that encode for a Bw4 epitope provide ligands for a KIR receptor called KIR3DL1. Indeed, a haplotype containing HLA-B*57 (HLA-Bw4) has previously been implicated with psoriasis [51]. HLA-E is a nonclassical class I molecule that provides a ligand for NKG2A/CD94, and NKG2C/CD94 receptors expressed by NK cells, and MICA encodes an antigen expressed in response to stress or particular pathological situations that activates NK cells through ligation of NKG2D [52]. An allele of MICA that possibly encodes for a soluble receptor, termed MICA A5.1, is associated with an increased risk of Psoriasis Vulgaris in a Chinese patient cohort [53]. Although there has been some controversy in the literature [6, 54], it seems likely that HLA-Cw6 constitutes the actual

risk variant rather than acting as a marker for a nearby gene that is responsible for the effects seen. HLA-C is particularly interesting from an NK cell perspective as all HLA-C alleles encode ligands for KIR expressed by NK cells [55].

Several loci outside of the MHC region have also been implicated in psoriasis. Some of these encode for molecules involved in skin and tissue functions, for example, within the epidermal differentiation complex, but many of them have known functions within the immune system. Among these are cytokines, defence molecules, and immune signalling components and regulators. These have been described in detail elsewhere [6, 35, 56] and this paper will focus only on those that relate to NK cells. The *IL-12B* gene that encodes the p40 subunit of both IL-12 and IL-23 has been identified by GWAS and replicates a previous candidate gene association study in the Japanese [57, 58]. The *IL-23A* gene encoding for the p19 subunit of IL-23 is also associated with psoriasis [59]. These p19 and p40 subunits combine to generate IL-23 cytokine that is known to promote differentiation of specialised T-cell (Th17) and NK cell (NK22) subsets [40]. Given that the *IL-23R* gene (receptor for IL-23) also associates with psoriasis, it seems that IL-12 or more likely, IL-23, plays a role in psoriasis. The role of IL-12 in NK cell biology is well established. It is known to activate NK cell functions, for example, cytotoxicity and promote secretion of IFN-γ [60]. A role for IL-23 has recently been shown in the generation of the novel NK cell subset, NK22 cells, that appear to have specialised functions in mucosal sites within the body [40, 61]. Two other loci (TNFAIP3 and TNIP1) identified by GWAS influence immune signalling [59]. It is thought that these gene products combine to inhibit NFκB signalling that is an important component in keratinocyte proliferation and differentiation in psoriasis. These molecules inhibit both TNF and Toll-like receptor signalling pathways.

5. Role of NK Cell Receptors in Psoriasis

The KIR genes are a family of genes found on human chromosome 19q13.4 [55]. They encode for membrane bound receptors that are expressed by NK cells and a subset of T-cells. These receptors interact with HLA class I antigen expressed on the surface of all nucleated cells. Some of the KIR receptors activate NK cells upon interactions with ligand and others inhibit NK cell functions. The activating receptors (termed 2DS or 3DS KIR) lack a functioning cytoplasmic tail but rather associate with adaptor molecules that transduce a positive signal to NK cells. In contrast, the inhibitory receptors (termed 2DL or 3DL KIR) have inherent inhibitory signalling capacity through Immuno Tyrosine-based Inhibitory Motifs (ITIMs) in their cytoplasmic tails. KIR haplotypes vary in terms of gene number, gene content, and allelic polymorphism such that there is huge genetic variation even within closely related ethnic groups [55, 62]. Significant progress has been made in terms of molecular typing to the extent that all genes can be identified by relatively straightforward PCR-SSP reactions [63]. However, studying the cellular expression and functions of these receptors is more difficult due to the fact that they are clonally expressed (i.e., individual NK cells will express different combinations of the KIR genes encoded for in their chromosomes) and that specific antibodies to individual KIR receptors have been difficult to generate given the high sequence similarities of receptors in their extracellular domains. Thus genetic analysis of KIR genes far supersedes our understanding of their biology [64].

HLA class I ligands for many of the KIR receptors have been described. Unlike the TCR that recognises individual alleles of HLA class I antigen (though the process of positive selection during development), NK cell receptors have evolved to recognise conserved epitopes of HLA class I receptors. Recognition of HLA-C by NK cells is particularly important as every HLA-C allele contains a motif recognised by a KIR receptor: HLA-C1 alleles have an NxxK motif at residues 77–80 of the α1 heavy chain that provides an epitope recognised by 2DL2, 2DL3, and 2DS2 receptors while HLA-C2 alleles have an SxxN motif at this same region that is recognised by 2DL1 and 2DS1 KIR receptors [55]. More recently this relatively straightforward delineation of specificities has blurred with the recognition that the 2DL2 receptor (that recognises HLA-C1) also weakly recognises HLA-C2 [65]. A subset of HLA-B genes encode a serological epitope termed Bw4 that provides a ligand for the 3DL1 and possibly the 3DS1 receptor [66]. Recognition of HLA-A gene products is different as it appears that a particular KIR receptor, 3DL2, has evolved to recognise individual HLA-A alleles (A3 and A11) and recognition is peptide dependent [67].

While HLA class I ligands for some activating KIR have been described as mentioned above, binding affinity of activating KIR for HLA class I is generally weaker than that of the inhibitory receptors. Recently, a systematic search of HLA class I allotypes for 2DS3, 2DS5 interactions failed to identify a ligand [68]. Together these facts have resulted in a growing consensus that perhaps HLA class I is not the primary ligand for activating KIR receptors. Possible ligands could include pathogen encoded molecules or endogenous molecules expressed under pathogenic conditions.

Molecular typing methods have provided tools for KIR gene association studies. KIR genes are good candidates for genetic associations with disease for a number of reasons: (1) genes of the immune system are often associated with the development or pathogenesis of disease; (2) KIR genes are polygenic and highly polymorphic; (3) KIR proteins interact with HLA class I gene products and HLA class I genes consistently provide the strongest associations with particular disease. KIR genes have been associated with the development or progression of disease in a number of clinical situations including infections, for example, HIV [69] and HCV infections [70] and noninfectious conditions, for example, recurrent miscarriage [71, 72]. It has become clear that the effects of KIR genes on disease may be subtle and may only manifest in the presence of cognate HLA class I ligand. As KIR genes and HLA class I genes segregate on different chromosomes, their presence together is relatively random. Thus disease association studies often need to investigate both KIR genes and HLA class I genes for interactions to become apparent.

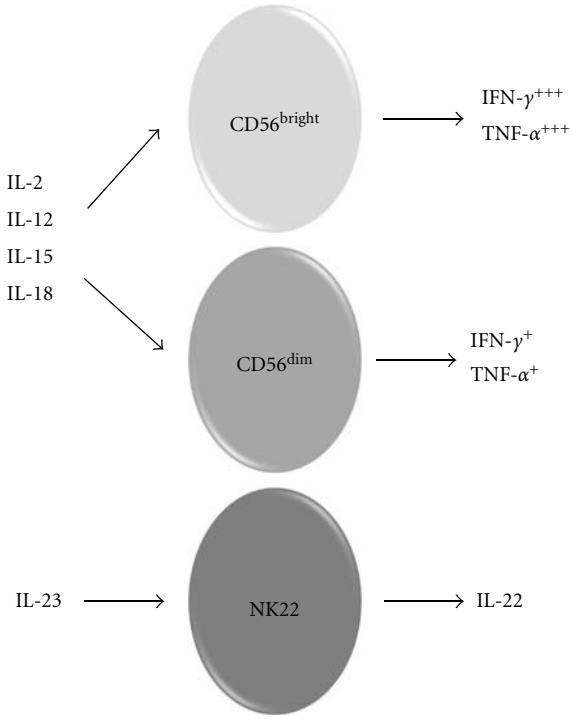

Figure 2: Subsets of NK cells secrete distinct cytokine profiles. Conventional NK cells, consisting of $CD56^{bright}$ and $CD56^{dim}$ subsets, are found in the general blood circulation. They are responsive to cytokines, with IL-2, IL-12, IL-15, and IL-18 being viewed as most important. These cytokines, some of which may act alone while others are only effective in as part of a milieu, activate effector functions such as secretion of cytokines including IFN-γ and TNF-α and degranulation of cytotoxic granules. In contrast, NK22 cells are a recently described subset of NK cells that are primarily resident in mucosal sites within the body. They differentiate towards the NK22 phenotype in response to IL-23 stimulation. NK22 cells secrete IL-22 cytokine (but not IL-17). IL-22 is known to be pathogenic in psoriasis.

Several studies have looked for KIR gene associations with the development of psoriasis and these have mainly focussed on Psoriasis Vulgaris. However, the data emerging do not provide a clear consensus with some studies reporting a significant association of 2DS1 with the development of disease while others find no KIR gene association. For the studies that did find a KIR gene association, 2DS1 has consistently been identified as an important locus. In brief, no KIR gene associations have been found in either Chinese [73] or US Caucasians [74]. Our preliminary analyses support these studies with no individual KIR gene associated with the development of psoriasis in an Irish cohort (S. Dunphy, data not shown). 2DS1 has been associated with the development of psoriasis is Swedish [75], Polish [76] and Japanese patients [77]. In the Japanese study, 2DL5 was also associated with the development of psoriasis. As the populations studied to date come from a variety of ethnic backgrounds, this is unlikely to account for the differences observed. Another factor to be considered is the size of the cohorts examined in terms of statistical power to demonstrate a result. The studies cited range from 96 patients and 50 controls at the lower end (Japanese cohort) to 237 patient and 372 controls at the higher end (Swedish cohort) and thus sample size may also contribute to differences seen. The frequency of 2DS1 within the control populations varies and this will also impact the ability to identify a role for 2DS1 is psoriasis. Therefore, additional highly powered studies are needed to resolve the role of 2DS1 in the development of psoriasis. However, it is interesting that the three studies that found a KIR gene association with psoriasis all identified 2DS1 as a locus of interest. 2DS1 is particularly attractive in this context as it is the only activating KIR receptor that has been shown to interact with HLA-Cw6 [68], which as previously mentioned, is the strongest genetic susceptibility locus identified to date in the development of psoriasis. A functional interaction between 2DS1 and HLA-Cw6 receptors may provide a biological rationale for NK cell involvement in psoriasis and thus, confirmation of 2DS1 as a susceptibility locus, is an important research question for NK cell biologists. It is worth noting that even if 2DS1 is confirmed other studies, the nature of KIR gene haplotype structure means that 2DS1 may be functioning as a marker for another nearby locus that is mediating the biological effect.

Psoriatic arthritis is a form of severe arthritis found in a subset of patients with psoriasis. The first study published on KIR genes in PsA identified two activating KIR genes, 2DS1 and 2DS2 associated with the development of psoriatic arthritis but only in the absence of their cognate HLA-C ligands in a US cohort [78]. The revised model proposed by the authors suggested that increasing susceptibility to psoriatic arthritis was determined by presence of more activating KIR genotypes (i.e., presence of activating KIR or lack of inhibitory KIR, in presence of ligand) [79]. A Swedish cohort found a trend for 2DS1 involvement in psoriatic arthritis but it did not reach statistical significance [75]. Finally, a second US cohort identified 2DS1 as a risk factor for the development of psoriatic arthritis among patients with psoriasis; however, numbers of patients in this analysis were low [74]. A single study looking at KIR genes in guttate psoriasis found no significant gene associations with development of disease [75].

Although GWAS studies have successfully identified many loci involved in psoriasis, it is clear that much of the genetic contribution to psoriasis remains to be identified. KIR genes may be among the "missing" genetic risk loci as it is unlikely that they would be identified on a genomewide scan of SNPs, given their complex haplotype variability and polymorphic nature.

6. Future Areas of Research

While there is evidence emerging to support a role for NK cells in psoriasis, more work needs to be done before we have a clearer picture of what that actual role might be. One issue that complicates this question is the variety of clinical patients cohorts that have been examined in the context of NK cells. Some groups have studied new onset Psoriasis Vulgaris while others look at guttate psoriasis, PsA

or the effect of treatment for psoriasis on NK cell function. While some of the KIR studies replicate an association of the 2DS1 gene with psoriasis, others have failed to do so. Thus, additional cohorts with adequate numbers and statistical power to address the relevance of this are required. In general, few of the studies in the literature have been replicated in other cohorts which makes it difficult to assess the overall role of NK cells in psoriasis. The field would greatly benefit from some basic NK cell studies in well-defined and characterised cohorts that have adequate numbers to confirm basic findings to date.

However, evidence from large GWAS studies and cell-based experimental systems have identified many potential molecules involved with NK cell biology that are associated with psoriasis including IL-12 and IL-23. Such analysis may lead to new exciting avenues of research, for example, a possible role for NK22 cells in psoriasis. From the GWAS studies, IL-23 is known to be important in psoriasis [57]. As previously described, it is the key cytokine responsible for the differentiation of the NK22 cell type that secretes IL-22 but not IL-17 cytokine (Figure 2) [40, 61]. NK22 cells are not found in peripheral blood but rather in mucosal/tissue sites within the body. It is tempting to speculate a role for NK22 cells particularly as IL-22 is known to be pathogenic in psoriasis. One could envision a dynamic interaction between NK cells that are localised to the skin by their chemokine receptor expression patterns and that differentiate towards NK22 cells in the presence of IL-23 cytokine that is secreted by keratinocytes. The NK22 cells could then secrete IL-22 which mediates its pathogenic effects. Alternatively, NK cells recruited to the skin may exhibit a more conventional NK cell phenotype and secrete IFN-γ and TNF-α that are known to play a role in psoriasis. If NK cells are involved, and much evidence supports that they are, it is as yet impossible to predict how these events are coordinated. Are NK cells normally resident in the skin activated to secrete factors that activate keratinocytes, or do activated keratinocytes recruit NK cells to the skin and activate them to secrete cytokines? Do both cell types participate in a positive feedback loop? In summary, evidence is emerging to support a role for NK cells in psoriasis but this field is in relative infancy compared to studies on other immune cells, for example, T-cells. More basic studies are required but early indications are that such studies will be fruitful and reveal new molecular mechanisms for NK cell involvement with initiation and progression of psoriasis.

References

[1] F. O. Nestle, D. H. Kaplan, and J. Barker, "Mechanisms of disease: psoriasis," *The The New England Journal of Medicine*, vol. 361, no. 5, pp. 444–509, 2009.

[2] M. A. Lowes, A. M. Bowcock, and J. G. Krueger, "Pathogenesis and therapy of psoriasis," *Nature*, vol. 445, no. 7130, pp. 866–873, 2007.

[3] M. Lebwohl, "Psoriasis," *The Lancet*, vol. 361, no. 9364, pp. 1197–1204, 2003.

[4] M. P. Schön and W. H. Boehncke, "Psoriasis," *The New England Journal of Medicine*, vol. 352, no. 18, pp. 1899–1912, 2005.

[5] Y. Liu, J. G. Krueger, and A. M. Bowcock, "Psoriasis: genetic associations and immune system changes," *Genes and Immunity*, vol. 8, no. 1, pp. 1–12, 2007.

[6] A. M. Bowcock and J. G. Krueger, "Getting under the skin: the immunogenetics of psoriasis," *Nature Reviews Immunology*, vol. 5, no. 9, pp. 699–711, 2005.

[7] A. L. Cameron, B. Kirby, W. Fei, and C. Griffiths, "Natural killer and natural killer-T cells in psoriasis," *Archives of Dermatological Research*, vol. 294, no. 8, pp. 363–369, 2002.

[8] A. L. Cameron, B. Kirby, and C. E. M. Griffiths, "Circulating natural killer cells in psoriasis," *British Journal of Dermatology*, vol. 149, no. 1, pp. 160–164, 2003.

[9] H. Bachelez, "Immunopathogenesis of psoriasis: recent insights on the role of adaptive and innate immunity," *Journal of Autoimmunity*, vol. 25, supplement, pp. 69–73, 2005.

[10] P. Di Meglio and F. O. Nestle, "The role of IL-23 in the immunopathogenesis of psoriasis," *F1000 Biology Reports*, vol. 2, no. 1, 2010.

[11] J. D. Bos, M. A. De Rie, M. B. M. Teunissen, and G. Piskin, "Psoriasis: dysregulation of innate immunity," *British Journal of Dermatology*, vol. 152, no. 6, pp. 1098–1107, 2005.

[12] A. T. Pietrzak, A. Zalewska, G. Chodorowska et al., "Cytokines and anticytokines in psoriasis," *Clinica Chimica Acta*, vol. 394, no. 1-2, pp. 7–21, 2008.

[13] O. Boyman, C. Conrad, G. Tonel, M. Gilliet, and F. O. Nestle, "The pathogenic role of tissue-resident immune cells in psoriasis," *Trends in Immunology*, vol. 28, no. 2, pp. 51–57, 2007.

[14] S. Peternel and M. Kaštelan, "Immunopathogenesis of psoriasis: focus on natural killer T cells," *Journal of the European Academy of Dermatology and Venereology*, vol. 23, no. 10, pp. 1123–1127, 2009.

[15] A. Koreck, A. Surányi, B. J. Szöny et al., "CD3+CD56+ NK T cells are significantly decreased in the peripheral blood of patients with psoriasis," *Clinical and Experimental Immunology*, vol. 127, no. 1, pp. 176–182, 2002.

[16] B. Bonish, D. Jullien, Y. Dutronc et al., "Overexpression of CD1d by keratinocytes in psoriasis and CD1d-dependent IFN-γ production by NK-T cells," *Journal of Immunology*, vol. 165, no. 7, pp. 4076–4085, 2000.

[17] A. Gilhar, Y. Ullmann, H. Kerner et al., "Psoriasis is mediated by a cutaneous defect triggered by activated immunocytes: induction of psoriasis by cells with natural killer receptors," *Journal of Investigative Dermatology*, vol. 119, no. 2, pp. 384–391, 2002.

[18] B. J. Nickoloff, B. Bonish, B. B. Huang, and S. A. Porcelli, "Characterization of a T cell line bearing natural killer receptors and capable of creating psoriasis in a SCID mouse model system," *Journal of Dermatological Science*, vol. 24, no. 3, pp. 212–225, 2000.

[19] B. J. Nickoloff, T. Wrone-Smith, B. Bonish, and S. A. Porcelli, "Response of murine and normal human skin to injection of allogeneic blood-derived psoriatic immunocytes: detection of T cells expressing receptors typically present on natural killer cells, including CD94, CD158, and CD161," *Archives of Dermatology*, vol. 135, no. 5, pp. 546–552, 1999.

[20] K. E. Nograles, B. Davidovici, and J. G. Krueger, "New insights in the immunologic basis of psoriasis," *Seminars in Cutaneous Medicine and Surgery*, vol. 29, no. 1, pp. 3–9, 2010.

[21] K. E. Nograles, L. C. Zaba, E. Guttman-Yassky et al., "Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways," *British Journal of Dermatology*, vol. 159, no. 5, pp. 1092–1102, 2008.

[22] C. Ottaviani, F. Nasorri, C. Bedini, O. de Pità, G. Girolomoni, and A. Cavani, "CD56brightCD16(-) NK cells accumulate in psoriatic skin in response to CXCL10 and CCL5 and exacerbate skin inflammation," *European Journal of Immunology*, vol. 36, no. 1, pp. 118–128, 2006.

[23] T. Carbone, F. Nasorri, D. Pennino et al., "CD56highCD16-CD62L- NK cells accumulate in allergic contact dermatitis and contribute to the expression of allergic responses," *Journal of Immunology*, vol. 184, no. 2, pp. 1102–1110, 2010.

[24] J. Skrzeczyńska-Moncznik, A. Stefańska, B. A. Zabel, M. Kapińska-Mrowiecka, E. C. Butcher, and J. Cichy, "Chemerin and the recruitment of NK cells to diseased skin," *Acta Biochimica Polonica*, vol. 56, no. 2, pp. 355–360, 2009.

[25] S. Parolini, A. Santoro, E. Marcenaro et al., "The role of chemerin in the colocalization of NK and dendritic cell subsets into inflamed tissues," *Blood*, vol. 109, no. 9, pp. 3625–3632, 2007.

[26] S. Aractingi, N. Briand, C. Le Danff et al., "HLA-G and NK receptor are expressed in psoriatic skin: a possible pathway for regulating infiltrating T cells?" *American Journal of Pathology*, vol. 159, no. 1, pp. 71–77, 2001.

[27] R. N. Cardili, T. G. Alves, J. C. O. C. Freitas et al., "Expression of human leucocyte antigen-G primarily targets affected skin of patients with psoriasis," *British Journal of Dermatology*, vol. 163, no. 4, pp. 769–775, 2010.

[28] M. López-Botet, F. Navarro, and M. Llano, "How do NK cells sense the expression of HLA-G class Ib molecules?" *Seminars in Cancer Biology*, vol. 9, no. 1, pp. 19–26, 1999.

[29] D. Ge, J. Fellay, A. J. Thompson et al., "Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance," *Nature*, vol. 461, no. 7262, pp. 399–401, 2009.

[30] A. Gilhar, R. Yan IV, B. Assy, S. Serafimovich, Y. Ullmann, and R. S. Kalish, "Fas pulls the trigger on psoriasis," *American Journal of Pathology*, vol. 168, no. 1, pp. 170–175, 2006.

[31] N. Yawalkar, S. Schmid, L. R. Braathen, and W. J. Pichler, "Perforin and granzyme B may contribute to skin inflammation in atopic dermatitis and psoriasis," *British Journal of Dermatology*, vol. 144, no. 6, pp. 1133–1139, 2001.

[32] L. Prpić, N. Štrbo, V. Sotošek, F. Gruber, E. R. Podack, and D. Rukavina, "Assessment of perforin expression in peripheral blood lymphocytes in psoriatic patients during exacerbation of disease," *Acta Dermato-Venereologica, Supplement*, no. 211, pp. 14–16, 2000.

[33] L. Prpić Massari, M. Kaštelan, G. Laškarin, G. Zamolo, D. Massari, and D. Rukavina, "Analysis of perforin expression in peripheral blood and lesions in severe and mild psoriasis," *Journal of Dermatological Science*, vol. 47, no. 1, pp. 29–36, 2007.

[34] C. L. Langrish, B. S. McKenzie, N. J. Wilson, R. De Waal Malefyt, R. A. Kastelein, and D. J. Cua, "IL-12 and IL-23: master regulators of innate and adaptive immunity," *Immunological Reviews*, vol. 202, pp. 96–105, 2004.

[35] R. P. Nair, J. Ding, K. C. Duffin et al., "Psoriasis bench to bedside: genetics meets immunology," *Archives of Dermatology*, vol. 145, no. 4, pp. 462–464, 2009.

[36] G. Piskin, R. M. R. Sylva-Steenland, J. D. Bos, and M. B. M. Teunissen, "In vitro and in situ expression of IL-23 by keratinocytes in healthy skin and psoriasis lesions: enhanced expression in psoriatic skin," *Journal of Immunology*, vol. 176, no. 3, pp. 1908–1915, 2006.

[37] L. S. Villadsen, J. Schuurman, F. Beurskens et al., "Resolution of psoriasis upon blockade of IL-15 biological activity in a xenograft mouse model," *Journal of Clinical Investigation*, vol. 112, no. 10, pp. 1571–1580, 2003.

[38] R. Ruckert, K. Asadullah, M. Seifert et al., "Inhibition of keratinocyte apoptosis by IL-15: a new parameter in the pathogenesis of psoriasis?" *Journal of Immunology*, vol. 165, no. 4, pp. 2240–2250, 2000.

[39] K. Wolk, H. S. Haugen, W. Xu et al., "IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN-γ are not," *Journal of Molecular Medicine*, vol. 87, no. 5, pp. 523–536, 2009.

[40] M. Colonna, "Interleukin-22-producing natural killer cells and lymphoid tissue inducer-like cells in mucosal immunity," *Immunity*, vol. 31, no. 1, pp. 15–23, 2009.

[41] M. Cella, A. Fuchs, W. Vermi et al., "A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity," *Nature*, vol. 457, no. 7230, pp. 722–725, 2009.

[42] K. Schroder, P. J. Hertzog, T. Ravasi, and D. A. Hume, "Interferon-γ: an overview of signals, mechanisms and functions," *Journal of Leukocyte Biology*, vol. 75, no. 2, pp. 163–189, 2004.

[43] M. A. Cooper, T. A. Fehniger, and M. A. Caligiuri, "The biology of human natural killer-cell subsets," *Trends in Immunology*, vol. 22, no. 11, pp. 633–640, 2001.

[44] F. Brandrup, M. Hauge, K. Henningsen, and B. Eriksen, "Psoriasis in an unselected series of twins," *Archives of Dermatology*, vol. 114, no. 6, pp. 874–878, 1978.

[45] D. L. Duffy, L. S. Spelman, and N. G. Martin, "Psoriasis in Australian twins," *Journal of the American Academy of Dermatology*, vol. 29, no. 3, pp. 428–434, 1993.

[46] M. Pisani and V. Ruocco, "'Twin' psoriasis in monozygotic twins," *Archives of Dermatology*, vol. 120, no. 11, pp. 1418–1419, 1984.

[47] A. Tiilikainen, A. Lassus, and J. Karvonen, "Psoriasis and HLA-Cw6," *British Journal of Dermatology*, vol. 102, no. 2, pp. 179–184, 1980.

[48] A. M. Bowcock, "Psoriasis genetics: the way forward," *Journal of Investigative Dermatology*, vol. 122, no. 6, pp. xv–xvii, 2004.

[49] R. P. Nair, P. Stuart, T. Henseler et al., "Localization of psoriasis-susceptibility locus PSORS1 to a 60-kb interval telomeric to HLA-C," *American Journal of Human Genetics*, vol. 66, no. 6, pp. 1833–1844, 2000.

[50] R. P. Nair, P. E. Stuart, I. Nistor et al., "Sequence and haplotype analysis supports HLA-C as the psoriasis susceptibility 1 gene," *American Journal of Human Genetics*, vol. 78, no. 5, pp. 827–851, 2006.

[51] B. J. Feng, L. D. Sun, R. Soltani-Arabshahi et al., "Multiple loci within the major histocompatibility complex confer risk of psoriasis," *PLoS Genetics*, vol. 5, no. 8, Article ID e1000606, 2009.

[52] A. Cerwenka and L. L. Lanier, "NKG2D ligands: unconventional MHC class I-like molecules exploited by viruses and cancer," *Tissue Antigens*, vol. 61, no. 5, pp. 335–343, 2003.

[53] L. Cheng, S. Z. Zhang, C. Y. Xiao et al., "The A5.1 allele of the major histocompatibility complex class I chain-related gene A is associated with psoriasis vulgaris in Chinese," *British Journal of Dermatology*, vol. 143, no. 2, pp. 324–329, 2000.

[54] S. Jenisch, E. Westphal, R. P. Nair et al., "Linkage disequilibrium analysis of familial psoriasis: identification of multiple disease-associated MHC haplotypes," *Tissue Antigens*, vol. 53, no. 2, pp. 135–146, 1999.

[55] C. Vilches and P. Parham, "KIR: diverse, rapidly evolving receptors of innate and adaptive immunity," *Annual Review of Immunology*, vol. 20, pp. 217–251, 2002.

[56] E. D. O. Roberson and A. M. Bowcock, "Psoriasis genetics: breaking the barrier," *Trends in Genetics*, vol. 26, no. 9, pp. 415–423, 2010.

[57] M. Cargill, S. J. Schrodi, M. Chang et al., "A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes," *American Journal of Human Genetics*, vol. 80, no. 2, pp. 273–290, 2007.

[58] Y. Tsunemi, H. Saeki, K. Nakamura et al., "Interleukin-12 p40 gene (IL12B) 3'-untranslated region polymorphism is associated with susceptibility to atopic dermatitis and psoriasis vulgaris," *Journal of Dermatological Science*, vol. 30, no. 2, pp. 161–166, 2002.

[59] R. P. Nair, K. C. Duffin, C. Helms et al., "Genome-wide scan reveals association of psoriasis with IL-23 and NF-κB pathways," *Nature Genetics*, vol. 41, no. 2, pp. 199–204, 2009.

[60] J. S. Orange and C. A. Biron, "An absolute and restricted requirement for IL-12 in natural killer cell IFN-γ production and antiviral defense. Studies of natural killer and T cell responses in contrasting viral infections," *Journal of Immunology*, vol. 156, no. 3, pp. 1138–1142, 1996.

[61] E. Vivier, H. Spits, and T. Cupedo, "Interleukin-22-producing innate immune cells: new players in mucosal immunity and tissue repair?" *Nature Reviews Immunology*, vol. 9, no. 4, pp. 229–234, 2009.

[62] K. J. Guinan, R. T. Cunningham, A. Meenagh et al., "Signatures of natural selection and coevolution between killer cell immunoglobulin-like receptors (KIR) and HLA class i genes," *Genes and Immunity*, vol. 11, no. 6, pp. 467–478, 2010.

[63] C. Vilches, J. Castaño, N. Gómez-Lozano, and E. Estefanía, "Facilitation of KIR genotyping by a PCR-SSP method that amplifies short DNA fragments," *Tissue Antigens*, vol. 70, no. 5, pp. 415–422, 2007.

[64] C. M. Gardiner, "Killer cell immunoglobulin-like receptors on NK cells: the how, where and why," *International Journal of Immunogenetics*, vol. 35, no. 1, pp. 1–8, 2008.

[65] A. K. Moesta, P. J. Norman, M. Yawata, N. Yawata, M. Gleimer, and P. Parham, "Synergistic polymorphism at two positions distal to the ligand-binding site makes KIR2DL2 a stronger receptor for HLA-C Than KIR2DL3," *Journal of Immunology*, vol. 180, no. 6, pp. 3969–3979, 2008.

[66] J. E. Gumperz, L. D. Barber, N. M. Valiante et al., "Conserved and variable residues within the Bw4 motif of HLA-B make separable contributions to recognition by the NKB1 killer cell-inhibitory receptor," *Journal of Immunology*, vol. 158, no. 11, pp. 5237–5241, 1997.

[67] P. Hansasuta, T. Dong, H. Thananchai et al., "Recognition of HLA-A3 and HLA-A11 by KIR3DL2 is peptide-specific," *European Journal of Immunology*, vol. 34, no. 6, pp. 1673–1679, 2004.

[68] A. K. Moesta, T. Graef, L. Abi-Rached, A. M. O. Aguilar, L. A. Guethlein, and P. Parham, "Humans differ from other hominids in lacking an activating NK cell receptor that recognizes the C1 epitope of MHC class I," *Journal of Immunology*, vol. 185, no. 7, pp. 4233–4237, 2010.

[69] M. P. Martin, Y. Qi, X. Gao et al., "Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1," *Nature Genetics*, vol. 39, no. 6, pp. 733–740, 2007.

[70] S. I. Khakoo, C. L. Thio, M. P. Martin et al., "HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection," *Science*, vol. 305, no. 5685, pp. 872–874, 2004.

[71] S. E. Hiby, R. Apps, A. M. Sharkey et al., "Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2," *Journal of Clinical Investigation*, vol. 120, no. 11, pp. 4102–4110, 2010.

[72] S. E. Hiby, L. Regan, W. Lo, L. Farrell, M. Carrington, and A. Moffett, "Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage," *Human reproduction*, vol. 23, no. 4, pp. 972–976, 2008.

[73] Y. T. Chang, C. T. Chou, Y. M. Shiao et al., "The killer cell immunoglobulin-like receptor genes do not confer susceptibility to psoriasis vulgaris independently in Chinese," *Journal of Investigative Dermatology*, vol. 126, no. 10, pp. 2335–2338, 2006.

[74] F. Williams, A. Meenagh, C. Sleator et al., "Activating killer cell immunoglobulin-like receptor gene KIR2DS1 is associated with psoriatic arthritis," *Human Immunology*, vol. 66, no. 7, pp. 836–841, 2005.

[75] S. J. Holm, K. Sakuraba, L. Mallbris, K. Wolk, M. Ståhle, and F. O. Sánchez, "Distinct HLA-C/KIR genotype profile associates with guttate psoriasis," *Journal of Investigative Dermatology*, vol. 125, no. 4, pp. 721–730, 2005.

[76] W. Łuszczek, M. Mańczak, M. Cisło et al., "Gene for the activating natural killer cell receptor, KIR2DS1, is associated with susceptibility to psoriasis vulgaris," *Human Immunology*, vol. 65, no. 7, pp. 758–766, 2004.

[77] Y. Suzuki, Y. Hamamoto, Y. Ogasawara et al., "Genetic polymorphisms of killer cell immunoglobulin-like receptors are associated with susceptibility to psoriasis vulgaris," *Journal of Investigative Dermatology*, vol. 122, no. 5, pp. 1133–1136, 2004.

[78] M. P. Martin, G. Nelson, J. H. Lee et al., "Cutting edge: susceptibility to psoriatic arthritis: influence of activating killer Ig-like receptor genes in the absence of specific HLA-C alleles," *Journal of Immunology*, vol. 169, no. 6, pp. 2818–2822, 2002.

[79] G. W. Nelson, M. P. Martin, D. Gladman, J. Wade, J. Trowsdale, and M. Carrington, "Cutting edge: heterozygote advantage in autoimmune disease: hierarchy of protection/susceptibility conferred by HLA and killer Ig-like receptor combinations in psoriatic arthritis," *Journal of Immunology*, vol. 173, no. 7, pp. 4273–4276, 2004.