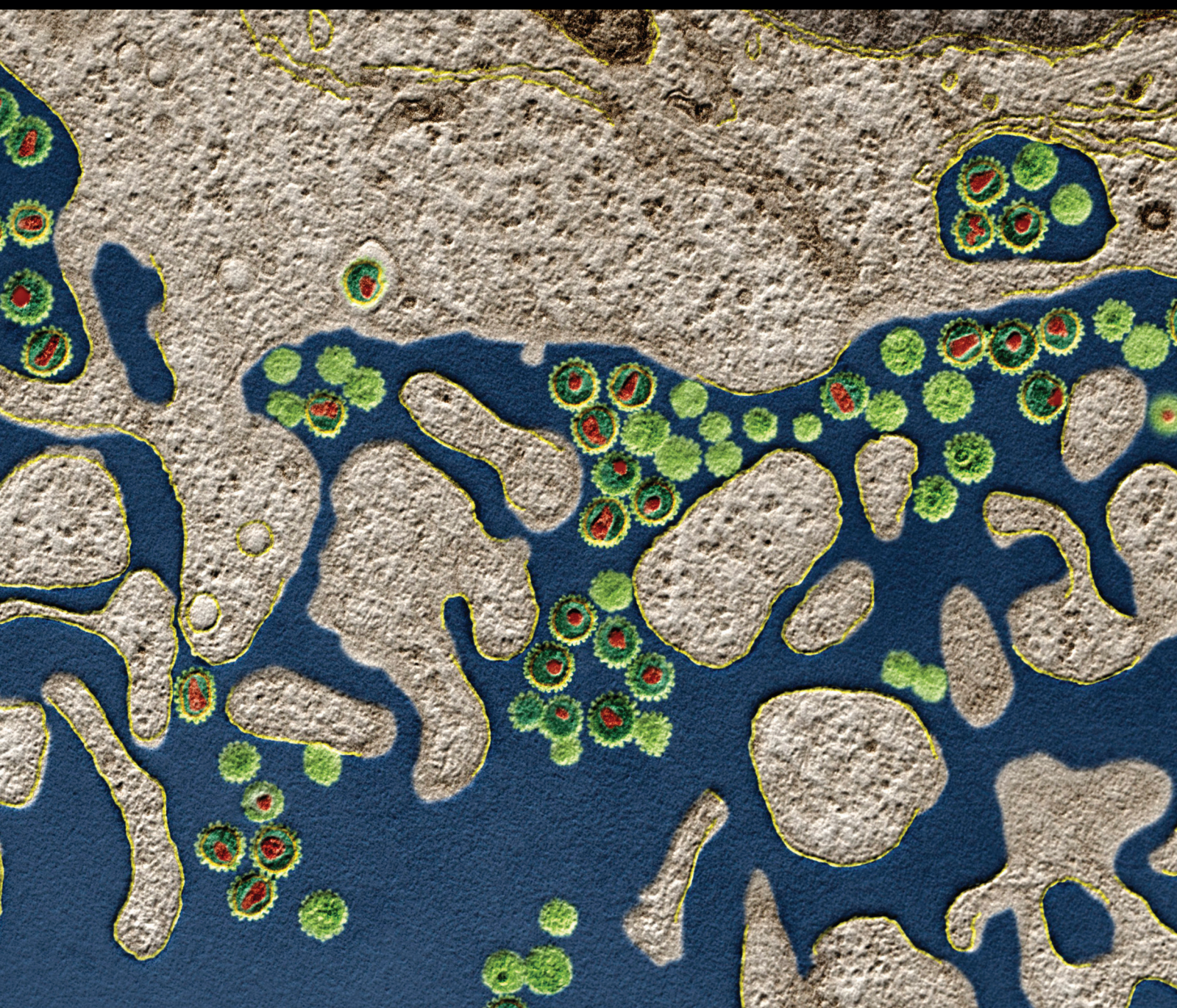
Journal of Immunology Research

Dendritic Cells in Tolerance and Immunity against Pathogens

Guest Editors: Silvia Beatriz Boscardin, Daniela Santoro Rosa, Alice O. Kamphorst, and Christine Trumpfheller



Dendritic Cells in Tolerance and Immunity against Pathogens

Journal of Immunology Research

Dendritic Cells in Tolerance and Immunity against Pathogens

Guest Editors: Silvia Beatriz Boscardin, Daniela Santoro Rosa, Alice O. Kamphorst, and Christine Trumpfheller

Editorial Board

Contents

Hindawi Publishing Corporation
Journal of Immunology Research
Volume 2016, Article ID 6438036, 2 pages
http://dx.doi.org/10.1155/2016/6438036

Editorial

Dendritic Cells in Tolerance and Immunity against Pathogens

Silvia Beatriz Boscardin,[1] Daniela Santoro Rosa,[2] Alice O. Kamphorst,[3] and Christine Trumpfheller[4]

[1]*Laboratory of Antigen Targeting to Dendritic Cells, Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo, 05508-000 São Paulo, SP, Brazil*
[2]*Department of Microbiology, Immunology and Parasitology, Federal University of São Paulo (UNIFESP/EPM), 04023-062 São Paulo, SP, Brazil*
[3]*Emory Vaccine Center and Department of Microbiology and Immunology, Emory University School of Medicine, 1510 Clifton Road Rm G214, Atlanta, GA 30322, USA*
[4]*Laboratory of Cellular Physiology and Immunology and Chris Browne Center, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA*

Correspondence should be addressed to Silvia Beatriz Boscardin; sbboscardin@usp.br

Received 21 March 2016; Accepted 23 March 2016

Dendritic cells (DCs) are a highly specialized population of antigen-presenting cells that play a key role in the induction of adaptive immune responses against different pathogens and in the maintenance of peripheral tolerance [1, 2]. Since their discovery by Ralph Steinman in the 70s [3–6], much has been learned about their ontogeny, migration, and antigen presentation capacities in lymphoid and nonlymphoid organs [7]. Advances in flow cytometry allowed the selection of surface markers that help distinguish different DC subsets [8]. In addition, the identification of many receptors has added information on how these cells sense the environment and respond to different stimuli [9]. The role of DCs in the induction of immune responses and/or in peripheral tolerance has been extensively studied, particularly in mice, using different models that include infectious diseases and cancer, but also autoimmune diseases. In addition, progress has been made in our understanding of human DC subsets.

In this special issue, a number of review articles will illustrate our current understanding of how DCs are affected in different contexts, ranging from inflammatory to more tolerogenic settings. For example, J. M. Motta and V. M. Rumjanek will discuss how different environments affect DC function. They focus especially on how DC function is modulated in the presence of tumors (a tolerogenic setting) or in the presence of organ transplantation (a more proinflammatory setting). The role of DCs following heart transplantation is also revised by M.-T. Dieterlen et al., when they additionally discuss in detail DC function in hypertension, atherosclerosis, and heart failure. On the other hand, S. Winning and J. Fandrey discuss how hypoxia modulates DC function. This is an important topic because antigen presentation normally takes place in organs and tissues that exhibit low oxygen tension, and we are just starting to recognize hypoxia as a key factor on the modulation of immune responses. On a more tolerant setting, we will learn from A. Steimle and J.-S. Frick how intestinal DCs interact constantly with different species of commensal bacteria and how these bacteria can regulate DC phenotype. DC function and tolerance break in systemic lupus erythematosus are addressed by X. Liao et al. Recent evidence suggests that DC activation by self-antigens contributes to tolerance breakdown and to the induction of lupus pathogenesis. The role of DCs in different contexts of infection is also the focus of three reviews in this special issue. N. A. Mabbott and B. M. Bradford address how prions may exploit conventional DCs to infect the host, while D. Feijó et al. and K. N. S. Amorim et al. focus on DC interactions with two different intracellular parasites. Interactions between DCs and parasites from the genus *Leishmania* are discussed by D. Feijó et al., while K. N. S. Amorim et al. overview the importance of DCs during *Plasmodium* infection and how

they sense different parasite components. Finally, I. G. Zizzari et al. specifically address how a DC receptor recognizes modified glycoproteins expressed by tumors and highlight the importance of antigen structure in the modulation of DC mediated immune responses.

New information is also provided by this special issue. T. Bertran et al. analyze interactions between *Gardnerella vaginalis* and human monocyte-derived DCs, while D. Clarke et al. study how DCs in contact with group B *Streptococcus* modulate activation of $CD4^+$ T cells.

In conclusion, this special issue highlights different functions of DCs in complex scenarios such as immunity and tolerance. Understanding how DCs help our immune system to deal with infections and to maintain the steady state is important, and such knowledge may be used in the design of better vaccines and in the treatment of autoimmune diseases.

Silvia Beatriz Boscardin
Daniela Santoro Rosa
Alice O. Kamphorst
Christine Trumpfheller

References

[1] R. M. Steinman and H. Hemmi, "Dendritic cells: translating innate to adaptive immunity," *Current Topics in Microbiology and Immunology*, vol. 311, pp. 17–58, 2006.

[2] R. M. Steinman and M. C. Nussenzweig, "Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 351–358, 2002.

[3] R. M. Steinman, J. C. Adams, and Z. A. Cohn, "Identification of a novel cell type in peripheral lymphoid organs of mice. IV. Identification and distribution in mouse spleen," *Journal of Experimental Medicine*, vol. 141, no. 4, pp. 804–820, 1975.

[4] R. M. Steinman and Z. A. Cohn, "Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution," *The Journal of Experimental Medicine*, vol. 137, no. 5, pp. 1142–1162, 1973.

[5] R. M. Steinman and Z. A. Cohn, "Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties in vitro," *The Journal of Experimental Medicine*, vol. 139, no. 2, pp. 380–397, 1974.

[6] R. M. Steinman, D. S. Lustig, and Z. A. Cohn, "Identification of a novel cell type in peripheral lymphoid organs of mice. 3. Functional properties in vivo," *Journal of Experimental Medicine*, vol. 139, no. 6, pp. 1431–1445, 1974.

[7] M. Merad, P. Sathe, J. Helft, J. Miller, and A. Mortha, "The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting," *Annual Review of Immunology*, vol. 31, pp. 563–604, 2013.

[8] K. Shortman and Y.-J. Liu, "Mouse and human dendritic cell subtypes," *Nature Reviews Immunology*, vol. 2, no. 3, pp. 151–161, 2002.

[9] K. Shortman and S. H. Naik, "Steady-state and inflammatory dendritic-cell development," *Nature Reviews Immunology*, vol. 7, no. 1, pp. 19–30, 2007.

Hindawi Publishing Corporation
Journal of Immunology Research
Volume 2016, Article ID 2926436, 11 pages
http://dx.doi.org/10.1155/2016/2926436

Review Article

Dendritic Cells and Their Multiple Roles during Malaria Infection

Kelly N. S. Amorim,[1] Daniele C. G. Chagas,[1] Fernando B. Sulczewski,[1] and Silvia B. Boscardin[1,2]

[1]*Laboratory of Antigen Targeting to Dendritic Cells, Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo, 05508-000 São Paulo, SP, Brazil*
[2]*National Institute for Science and Technology in Vaccines, 31270-910 Belo Horizonte, MG, Brazil*

Correspondence should be addressed to Silvia B. Boscardin; sbboscardin@usp.br

Received 26 November 2015; Accepted 6 March 2016

Academic Editor: Jacek Tabarkiewicz

Dendritic cells (DCs) play a central role in the initiation of adaptive immune responses, efficiently presenting antigens to T cells. This ability relies on the presence of numerous surface and intracellular receptors capable of sensing microbial components as well as inflammation and on a very efficient machinery for antigen presentation. In this way, DCs sense the presence of a myriad of pathogens, including *Plasmodium* spp., the causative agent of malaria. Despite many efforts to control this infection, malaria is still responsible for high rates of morbidity and mortality. Different groups have shown that DCs act during *Plasmodium* infection, and data suggest that the phenotypically distinct DCs subsets are key factors in the regulation of immunity during infection. In this review, we will discuss the importance of DCs for the induction of immunity against the different stages of *Plasmodium*, the outcomes of DCs activation, and also what is currently known about *Plasmodium* components that trigger such activation.

1. Introduction

Malaria is the most important protozoan parasitosis in humans. It afflicts millions of people annually causing an expressive burden, mainly in tropical countries. *Plasmodium* has a very complex life cycle, and its different stages alternate between the vertebrate host and the mosquito. The vertebrate infective stages are able to move around in the skin but also traverse and infect cells in tissues. During this journey, *Plasmodium* interacts with DCs that are responsible for the initiation of adaptive immune responses. The interactions among DCs and the parasite are complex and may shape the outcome of the anti-*Plasmodium* immune responses.

2. Dendritic Cells and Their Role in the Induction of Immune Responses

DCs are a distinct lineage of mononuclear phagocytic cells specialized in antigen presentation. They show excellent ability to capture, process, and present antigens to T cells [1], directly activate B cells [2], and are also involved in the amplification of innate immune responses, such as activation of NK cells [3, 4]. Once in contact with a pathogen, DCs undergo a process known as maturation that culminates with efficient antigen presentation and cytokine production. Cytokines produced by DCs become part of the microenvironment that induces immune responses capable of stimulating the development of effector T lymphocytes [5]. In addition, DCs are involved in tolerance development in the thymus by negative selection of autoreactive lymphocytes [6] and in the periphery, where they present self-antigens in the absence of inflammation [7]. This entire range of DC functions is associated with their ability to recognize pathogen- or damage-associated molecular patterns (PAMPs or DAMPs, resp.) through pattern recognition receptors (PRRs) [8].

Different classes of PRRs were discovered in the last decades and include membrane anchored receptors such as toll-like receptors (TLRs) [9] and C-type lectin receptors (CLRs) [10], besides the cytoplasmic nucleotide-binding oligomerization domain- (Nod-) like receptors (NLRs), RIG-I-like

receptors (RLRs), and AIM-2-like receptors [11, 12], as well as a family of enzymes that function as intracellular sensors of nucleic acids, including OAS proteins and cGAS [12]. These PRRs are capable of triggering complex intracellular signals that stimulate DC maturation, increase the expression of major histocompatibility complex (MHC) and costimulatory molecules, and promote proinflammatory cytokines expression [13, 14]. Thus, in a context of infection and inflammation, DCs can identify the presence of pathogens through PRRs and induce adaptive immune responses [13].

DCs can be subdivided into different subsets based on the expression of different surface molecules (Figure 1). Human and mouse DCs normally express CD45, CD11c, and MHC class II (MHCII). In mice, the CD11c and MHCII molecules are expressed in all DC subsets with different intensities, and other markers such as CD11b, CD8α, CD103, and B220 can be used to distinguish the subsets even further. DCs expressing B220 and intermediate amounts of CD11c are known as plasmacytoid dendritic cells (pDCs). DCs expressing high levels of CD11c are known as conventional dendritic cells (cDCs) or myeloid dendritic cells (mDCs) and can be subdivided into $CD8\alpha^+CD11b^-$ and $CD8\alpha^-CD11b^+$ in lymphoid tissues and $CD103^+CD11b^-$, $CD103^+CD11b^+$, and $CD103^-CD11b^+$ in nonlymphoid tissues [15]. Langerhans cells (LCs) are DCs that populate the epidermal layer of skin and, different from cDCs, undergo a unique differentiation process [16].

The $CD8\alpha^+CD11b^-$ cDCs are efficient in antigen capture and presentation to $CD4^+$ T cells in the context of MHCII molecules but have been mainly associated with antigen presentation to $CD8^+$ T cells [17]. They are also able to cross-present exogenous antigens in MHC class I (MHCI) molecules, promoting activation of $CD8^+$ T cells [18]. On the other hand, $CD8\alpha^-CD11b^+$ DCs are extremely efficient in presenting antigens to $CD4^+$ T cells, polarizing them to Th2 or Th17 that in turn help B cells to produce antibodies [19].

As mentioned above, cDCs can reside in lymphoid and nonlymphoid organs. Normally, cDCs residing in nonlymphoid organs such as skin, lungs, or gut are more frequently in contact with antigens derived from pathogens. They capture antigens in the periphery and then move to lymphoid organs, where they present them to T cells. During migration, DCs mature and are able to prime lymphocytes, initiating the adaptive immune response [20]. Evidence has shown that the $CD103^+$ DCs are the nonlymphoid organ counterparts of the $CD8\alpha^+$ DCs, as they have a similar expression profile and can also efficiently perform cross-presentation and $CD8^+$ T cell activation [15, 21].

On the other hand, pDCs express lower levels of MHCII and are much less efficient in inducing proliferation of T lymphocytes [22, 23]. However, during inflammation pDCs can be activated and differentiated into a specific DC subtype in spleen with higher capacity to induce T cell activation [24–26].

There are relatively fewer studies on human DCs when compared to mice, mainly because of the difficulty in isolating them from human tissues. In this way, most of our knowledge involves skin and blood DCs [15]. As in mice, human DCs can be divided into mDCs (or cDCs) and pDCs. In the blood and in lymphoid tissues, mDCs are $CD3^-CD14^-CD19^-CD20^-CD56^-$HLA-$DR^+CD11c^+$ [27] and can be further subdivided into two subsets that express CD1c (BDCA1) or CD141 (BDCA3) [28]. On the other hand, pDCs are $CD3^-CD14^-CD19^-CD20^-CD56^-$HLA-$DR^+CD11c^+CD303(BDCA2)^+CD304(BDCA4)^+$ [29]. In the skin, three populations can be found: $CD1a^+CD14^-$ DCs, $CD1a^-CD14^+$ DCs, and LCs [15, 30]. Their functions in the steady state or during inflammatory/infectious diseases are beginning to be elucidated only recently [31].

3. Malaria

Malaria is an infection caused by protozoa that belong to the phylum Apicomplexa, genus *Plasmodium*. This unicellular parasite is transmitted to humans through the bite of an infected mosquito. Approximately 200 million cases of this disease are reported annually causing half a million deaths. Most of the deaths occur among children living in Africa. Malaria is prevalent in tropical and subtropical regions and is currently endemic in parts of America and parts of Asia and in Sub-Saharan Africa. In 2013, 97 countries reported endemic malaria [32].

There are five species of *Plasmodium* that infect humans: *P. falciparum*, *P. vivax*, *P. malariae*, *P. knowlesi*, and *P. ovale* [32]. The parasitic cycle begins when infected mosquitoes transfer sporozoites (the infective forms of *Plasmodium*) during the blood meal. These forms can remain in the skin for hours, slowly reaching circulation [33, 34]. In the circulatory system, sporozoites are carried to the liver, where they invade and replicate within hepatocytes [35]. Replication gives rise to merozoites that are released from the liver in structures named merosomes and can subsequently invade erythrocytes [36]. This phase of the cycle is known as erythrocytic phase and is when malaria symptoms start. At this stage, schizogony is repeated at specific intervals depending on the species and the febrile seizures correlate with the release of merozoites in the circulation. After a few days, some merozoites that infect erythrocytes give rise to male and female gametocytes that once taken up by the mosquito continue the cycle in the invertebrate host.

Severe cases of malaria are generally caused by *P. falciparum*. Infection with this parasite may progress to cerebral malaria, and infected individuals often present neurological symptoms such as convulsions and coma. In addition, patients with severe malaria may also present abnormal posture, respiratory syndrome, severe anemia, and multiple organ failure [37, 38]. The fact that severe malaria is usually associated with *P. falciparum* may be related to the potential of this species to produce hyperparasitemia. On the other hand, *P. falciparum* is the only species that clearly produces alterations in the microcirculation, allowing the parasite to escape destruction in the spleen. For example, erythrocytes infected with *P. falciparum* have the ability to adhere to the microvasculature. This phenomenon is known as cytoadherence and is mediated by molecules expressed by the infected erythrocyte that are able to bind to a series of endothelial receptors [39], such as CD36 and ICAM-1 [40, 41]. In addition, infected erythrocytes are able to bind to other

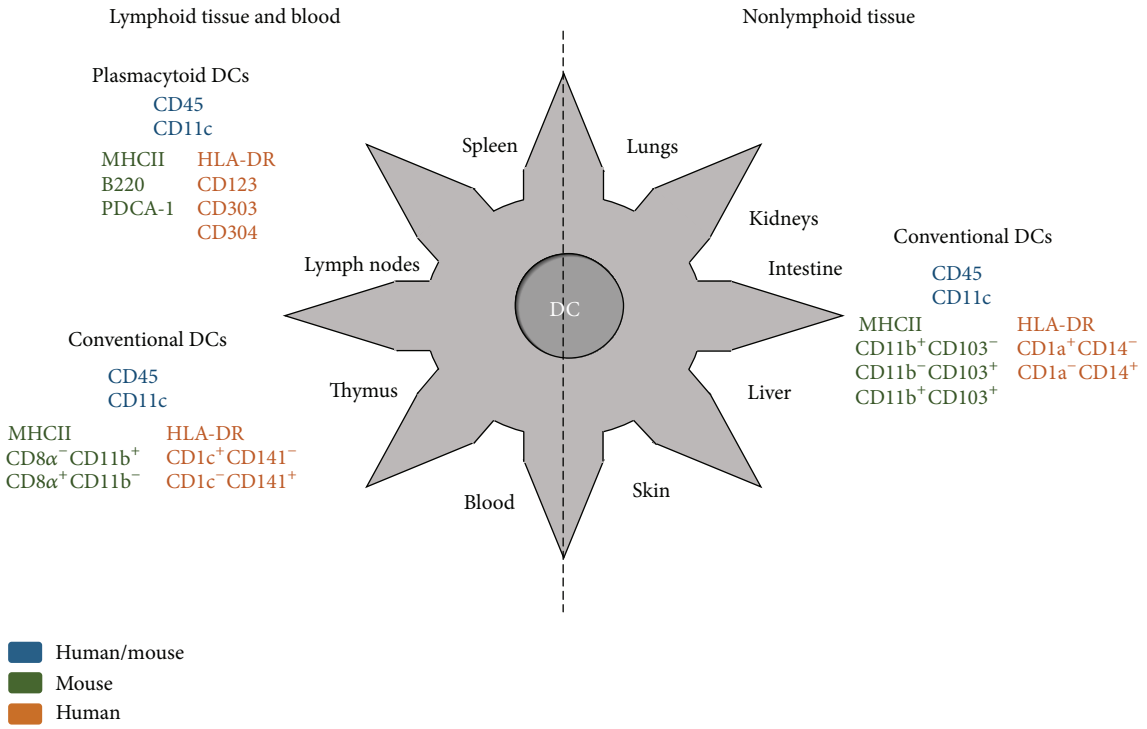


Figure 1: Most common DC surface markers used to differentiate conventional from plasmacytoid DCs and lymphoid tissue/blood from nonlymphoid tissue resident DCs. The colors indicate if a specific marker is expressed only in humans (orange), only in mice (green), or in both (blue) either in humans or in mice. Langerhans cells (LCs) are not represented. Based on [15, 125, 126].

infected and also noninfected erythrocytes, in a phenomenon known as rosetting. In this case, there is formation of cell aggregates that also interfere with the microcirculation [42].

The immunologic memory generated during infection with *Plasmodium* spp. is most often transient and restricted to patients living in endemic areas due to frequent exposure to the parasite by bites of infected mosquitoes [43]. In other words, naturally acquired immunity is not sterilizing and requires the persistence of the parasite to maintain the population of memory cells [44]. Several evidences suggest that naturally acquired protective immunity against malaria is obtained after successive infections [45]. Children intensely exposed to transmission have successive clinical episodes of malaria. With increasing age, clinical symptoms are less pronounced, although individuals may have high blood parasitemia [46]. In general, naturally acquired immunity is partly strain specific and primarily leads to a reduction of mortality rates and incidence of complications and later to a decrease in the incidence of disease. Finally, this naturally acquired immunity leads to a drop in parasitemia to low or even undetectable levels by conventional detection methods [47].

There is evidence that protective immune responses against malaria (sporozoites or blood stages) are initiated when antigen-presenting cells, DCs or macrophages, internalize the parasite and process and present its antigens to T cells via MHCI (through cross-presentation) or MHCII in a proinflammatory environment in which IL-12, TNFα, and IFNγ are produced. During the blood stage infection, it was shown that these cytokines act synergistically activating macrophages to produce reactive oxygen species (ROS) and nitrogen leading to parasite death [48, 49]. $CD8^+$ T cells have also been implicated in the protective immune response against sporozoites of *Plasmodium* [50, 51]. Several studies have shown that the $CD8^+$ T cells play a vital role in immunity against the preerythrocytic phase of *Plasmodium* both in mice [52] and in humans [53]. $CD8^+$ T cells eliminate infected hepatocytes and are capable of producing TNFα and IFNγ [54]. The role of $CD4^+$ T cells during *Plasmodium* infection has also been extensively studied in humans and mice. They seem to be particularly important during the erythrocytic phase, when IFNγ producing $CD4^+$ T cells are required for the elimination of iRBCs. In addition, follicular helper T (Tfh) cells were also shown to be pivotal for the activation of antibody producing B cells [55].

Our understanding of the immunity and immunoregulation that develop during malaria is still incomplete. DCs act in the immune response to activate and/or regulate production of proinflammatory or regulatory cytokines that may have fundamental roles in regulating the acquisition of protection or in the exacerbated response observed in severe malaria patients. In the following sections, we will discuss what is known about DC participation during *Plasmodium* infection. Much of this knowledge was generated using mouse models that can only be infected with murine *Plasmodium* species. Despite that limitation, mouse models have helped us to understand how DCs influence the anti-*Plasmodium* response.

4. Role of Dendritic Cells during *Plasmodium* Infection

DCs ability to present antigens during malaria infection was recently reviewed [48]. As mentioned above, DCs are responsible for T cell priming and thus regulate the development of adaptive immune responses [56]. DC function has been extensively studied during infection with different species of *Plasmodium*, and, in some aspects, contradictory data was obtained. The reasons for such contradictions could be ascribed to the use of different *Plasmodium* species and stages, as well as differences in the DC activation status. We will discuss the data in more detail in the sections ahead.

4.1. DCs during Acute Plasmodium Infection in Humans. The first study analyzing DC function directly in the context of *P. falciparum* infection was reported by Urban et al. These authors incubated monocyte-derived DCs with infected red blood cells (iRBCs) and showed that they were able to bind to human DCs and inhibit their maturation, reducing their ability to stimulate T cell responses [57]. These results were questioned by Elliott et al. when they showed that in fact inhibition of DC maturation was only obtained when iRBCs were used in a 100 : 1 iRBC : DC ratio and was not contact dependent. In addition, when a lower dose of iRBC (10 : 1 ratio) was used, DCs matured efficiently and activated autologous T cell proliferation [58]. In a field study with Kenyan children, BDCA3$^+$ DCs were significantly increased during acute infection, while CD1c$^+$ DC numbers were unaltered when compared to healthy individuals [59, 60]. In addition, an association between increased numbers of circulating BDCA3$^+$ DCs and severe human malaria was also observed [60, 61].

Although more prevalent, infection with *P. vivax* is more benign and less studied. However, a few studies were performed in an attempt to examine DC status and function during acute and symptomatic *P. vivax* infection. For example, the numbers of pDCs (HLA-DR$^+$CD123$^+$) and mDCs (HLA-DR$^+$CD11c$^+$) were evaluated in infected individuals from Thailand and Brazil, and a decrease in the mDCs/pDCs ratio was observed in both studies [62, 63]. In addition, 1/3 of the *P. vivax*-infected Brazilian patients showed low surface expression of CD86 [62]. A similar reduction in CD86 levels, as well as in CD83 and HLA-DR, was observed in Indonesian patients infected with *P. falciparum* and *P. vivax* [64]. This reduction correlated with an increase in DCs spontaneous apoptosis and impairment in their ability to capture, mature, and present antigens to T cells [64]. Interestingly, when patent asymptomatic patients were studied, HLA-DR expression was preserved in groups infected with either *P. vivax* or *P. falciparum* [65]. This result may indicate that DC function is preserved in patients that are infected but do no present symptoms, suggesting that functional DCs are important for the maintenance of clinical, but not parasitological, immunity.

4.2. Are DCs Capable of Inducing T and B Cell Responses during Plasmodium Infection? Human and murine species of *Plasmodium* have been used in studies designed to harness the participation of DCs in the induction of immunity against the preerythrocytic stages. DCs pulsed with sporozoite extracts were shown to elicit specific killing of *P. vivax* exoerythrocytic stages within infected hepatocytes [66], while DCs pulsed with a well-characterized CD8$^+$ T cell epitope derived from the *P. yoelii* circumsporozoite protein reduced the liver burden in BALB/c mice after a sporozoite challenge [67]. This CD8$^+$ T cell response was abrogated when DCs were depleted *in vivo* [68]. Moreover, after an infectious mosquito bite, the CD8α^+CD11b$^-$ DCs located in the draining lymph nodes were shown to be the DC subset responsible for CD8$^+$ T cell priming *in vivo* [69, 70] (Figure 2). In the *P. berghei* model, DCs pulsed with irradiated sporozoites were able to similarly prime central memory CD8$^+$ T cells when compared to DCs primed with untreated sporozoites. However, irradiation enhanced sporozoites' ability to prime effector CD8$^+$ T cells capable of producing IFNγ. In this particular study, the fine specificity of CD8$^+$ T cells was not evaluated [71]. A more detailed study showed that the two major splenic DCs subsets (CD8α^+CD11b$^-$ and CD8α^-CD11b$^+$) induced IFNγ producing CD8$^+$ T cells specific for the circumsporozoite protein, the major sporozoite surface protein [72]. In addition, the CD8$^+$ T cell protective response against a genetically modified *P. yoelii* strain was shown to be dependent on effective DC maturation obtained through CD40 signaling [73]. The fact that DCs are able to induce CD8$^+$ T cell responses against antigens expressed in the preerythrocytic stage seems undisputable. However, an observation made by Ocaña-Morgner et al. added another layer of complexity to the picture. These authors observed that DCs from mice previously infected with *P. yoelii* strain 17XNL and undergoing erythrocytic cycle presented an immature phenotype and were unable to initiate CD8$^+$ T cell responses to subsequent liver-stage antigens [74]. An unidentified soluble factor released by the iRBC seemed to be responsible for the inhibition of DCs maturation [75]. Cross-presentation to CD8$^+$ T cells was also inhibited during active *P. berghei* blood infection [76] and was mainly dependent on the CD8α^+CD11b$^-$ DCs subset [77]. Taken together, these results suggest that blood stage infection with *Plasmodium* can impair the development of an effective CD8$^+$ T cell response against the liver stages, which then could clear the parasite upon reinfection.

The role of DCs in the activation of CD4$^+$ T cell responses was also evaluated during *P. yoelii* infection (Figure 2). DCs derived from infected mice (day 6) presented higher expression of the surface costimulatory molecules CD80 and CD40 and were able to efficiently present antigens to CD4$^+$ T cells that in turn produced higher levels of IL-2, IFNγ, and TNFα. Production of these cytokines required DC-derived IL-12 [78, 79].

Infections with *P. chabaudi* have also shed light on the role of DCs during malaria. *In vitro* studies showed that *P. chabaudi* schizonts induced bone marrow-derived DCs to express MHCII and costimulatory molecules and to produce IL-6, IL-12, and TNFα [80]. An *in vivo* study showed that DCs migrated from the marginal zone of the spleen into the T cell area within 5 days after infection, and by day 7 an increase in costimulatory molecules was observed [81]. The role of CD8α^+CD11b$^-$ and CD8α^-CD11b$^+$ DCs subsets in

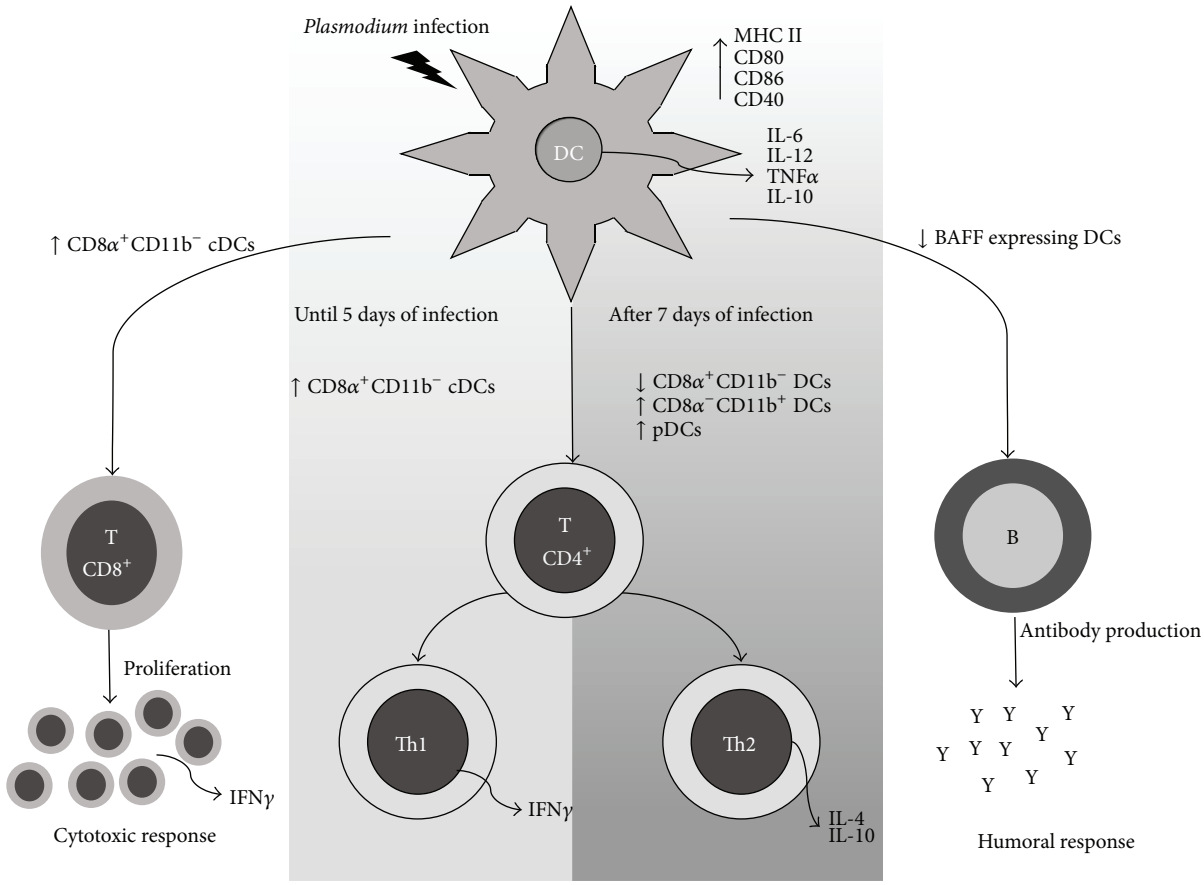


Figure 2: *Plasmodium* infection activates different DCs subsets. Infection with different stages of *Plasmodium* activates DCs that in turn start producing proinflammatory (IL-6, IL-12, and TNFα) but also regulatory (IL-10) cytokines. The CD8α^+CD11b$^-$ DCs are responsible for priming CD8$^+$ T cell responses against intrahepatic forms, while both cDCs subsets (CD8α^+CD11b$^-$ and CD8α^+CD11b$^+$) play a role in the activation of CD4$^+$ T cell responses that can lead to an inflammatory or regulatory outcome, depending on the timing of infection. pDCs also play a role in the induction of a more regulatory CD4$^+$ T cell response. The number of BAFF expressing DCs is reduced during *Plasmodium* infection, and that may reduce their ability to support B cell differentiation directly. Based on [69, 70, 78–80, 82, 85, 86, 91].

antigen presentation and specific CD4$^+$ T cell activation was also investigated. Despite the fact that both subsets induced IFNγ production, only the CD8α^-CD11b$^+$ DCs isolated at the infection peak (day 7) were able to induce proliferation of *Plasmodium*-specific Tg CD4$^+$ T cells and considerable amounts of IL-4 and IL-10 [82], indicating that this subset could be responsible for the switch in the balance from the proinflammatory Th1 response seen in the first few days to a more pronounced Th2 response. However, on days 10 and 13 after *P. chabaudi* infection, the CD8α^-CD11b$^+$ DCs were no longer able to induce CD4$^+$ T cell proliferation or cytokine production due to downregulation of costimulatory molecules and IL-12 expression [83]. Nevertheless, the *in vivo* CD4$^+$ T cell response later recovers and the mice are able to control infection, and at this stage antibodies seem to play an important role in the parasitemia control [55, 84]. pDCs were also studied in this model and, despite an increase in their numbers during infection, they played no role in CD4$^+$ T cell activation or in the control of infection [85]. On the other hand, in a *P. yoelii* model of infection, pDCs numbers increased by day 6 and remained high until at least day 14, and they were able to induce IL-10-expressing CD4$^+$ T cells [86]. Taken together the results discussed above suggest that different DC subsets have different functions during the blood stage *Plasmodium* infection, with cDCs involved in the induction of the proinflammatory response and pDCs accounting for a more balanced response at a later stage (Figure 2).

As reported for *P. falciparum* [57] and *P. yoelii* [74], DCs were also able to bind and internalize *P. chabaudi* iRBC. This phenomenon was shown to be partially dependent on actin polymerization, and the iRBCs uptake was again associated with increased expression of MHCII and costimulatory molecules, IL-12 production, and stimulation of CD4$^+$ T cell proliferation and IFNγ production [87]. Moreover, a recent study using intravital microscopy in mice showed that splenic DCs not only interact with CD4$^+$ T cells in T cell rich areas and in the red pulp but also very actively phagocytose iRBCs, contributing directly to their elimination during acute infection [88].

The function of DCs in the development of cerebral malaria was also assessed using a model of *P. berghei*

ANKA strain infection in C57BL/6 mice. In this setting, cDCs were shown to play a major role in the induction of cerebral malaria [89]. A more detailed study showed that the CD8α^+ and CD103$^+$ DCs subsets are essential to induce the pathogenic CD8$^+$ T cells responsible for lethal brain inflammation during *P. berghei* ANKA infection [60]. A recent set of experiments showed that the numbers of cDCs and pDCs were drastically reduced during *P. berghei* ANKA infection, through a mechanism that involved activation of caspase-3 and induction of DC apoptosis. In this particular case, the function of the remaining DCs was not evaluated [90].

DCs also play an important role in direct B cell activation (Figure 2). This happens because they produce a cytokine known as B cell activating factor (BAFF) that enhances B cell differentiation and survival [2]. During *P. yoelii* infection, there is a decrease in the percentage of DCs expressing BAFF, resulting in a reduction of their ability to support memory B cell differentiation into antibody secreting cells [91].

Finally, besides T and B cells, DCs are also able to interact with innate immune cells such as NK and $\gamma\delta$ T cells during *Plasmodium* infection. The depletion of NK cells during *P. berghei* ANKA infection led to a significant reduction in DC-mediated CD8$^+$ T cell priming but did not affect CD4$^+$ T cells. It seems that NK cells stimulate DCs to produce IL-12 that in turn is required for optimal T cell priming. The effect of DCs on NK cell function was also evaluated, and DC depletion reduced NK cell-mediated IFNγ responses to this *Plasmodium* species [92]. In the same way, $\gamma\delta$ T cells can also communicate with DCs and do so when they express CD40L and produce IFNγ that in turn enhance DC activation [49].

The data discussed above indicates that DCs may play distinct roles during *Plasmodium* infection, promoting either activation of protective immune responses or exacerbation of pathology.

4.3. How Do DCs Recognize Plasmodium Components? The expression of TLRs on DCs was studied in patients infected with *P. falciparum*. Patients with mild and severe forms of the disease displayed increased surface expression of TLR2 and TLR4 on mDCs and decreased intracellular expression of TLR9 on pDCs, when compared to healthy controls [93]. Despite this decreased TLR9 expression on pDCs, another study showed that the TLR9-MyD88 signaling pathway was required for pDC activation upon stimulation using schizonts or soluble schizont extracts [94]. The possible ligand for this pathway will be discussed below. A study using *P. chabaudi* infected mice showed that DCs from MyD88 knockout mice, but not from TLR2, TLR4, TLR6, TLR9, or CD14 knockout mice, were unable to produce proinflammatory cytokines and induce CD4$^+$ T cell responses [95]. This result indicates that the adaptor molecule MyD88 is required, but different ligands may be signalizing through different TLRs.

As mentioned before, DCs express different classes of PRRs capable of recognizing PAMPs derived from a vast array of pathogens [8]. In the case of *Plasmodium*, three PAMPs have been more extensively studied: hemozoin, immunostimulatory nucleic acid motifs, and glycosylphosphatidylinositol (GPI) anchors [96].

When *Plasmodium* invades the RBC, it degrades hemoglobin as a source of amino acids, which in turn releases heme that is potentially toxic. To survive, the parasite detoxifies heme into hemozoin using an enzyme named Heme Detoxification Protein (HDP) [97]. Contrasting results have been obtained and the role of hemozoin is still a matter of debate. Initially, hemozoin was shown to be present inside macrophages and circulating monocytes during *P. falciparum* infection, reducing their ability to phagocytose other particles or generate oxidative burst [98]. Human monocytes loaded with hemozoin and then *in vitro* differentiated into DCs presented impaired surface expression of MHCII and costimulatory molecules [99, 100], while monocyte-derived DCs incubated with synthetic hemozoin upregulated costimulatory molecules and released proinflammatory (IL-6) and anti-inflammatory (IL-10 and TNFα) cytokines, but not IL-12, leading to suboptimal T cell activation [101, 102]. In a *P. chabaudi* model, hemozoin-containing DCs were unable to fully activate T cells that in consequence did not cluster or migrate to lymphoid organ follicles [103, 104].

Different results showed that *P. falciparum* iRBC hemozoin, but not -hematin (a synthetic hemozoin), was able to induce human monocyte-derived DCs to upregulate costimulatory molecules (CD83, CD86, and CD1a) and produce IL-12 [105]. A follow-up study by the same group showed that this effect was dependent on TLR9 activation followed by MyD88 signaling but independent of TLR2, TLR4, TLR7, or TRIF [106]. In contrast with these authors, Parroche et al. showed that hemozoin is not a direct ligand for TLR9; instead it functions as a carrier for plasmodial DNA that is phagocytosed by DCs and carried to intracellular compartments [107]. Finally, Wu et al. argued that hemozoin was not a TLR9 ligand for DCs and did not function as a DNA carrier into cells. Instead, their results showed that a protein-DNA complex was the parasite's component responsible for the DC activation through TLR9 signaling. Protein-DNA complex formation was essential for the entry of parasite DNA into DCs leading to TLR9 recognition [108]. In addition, hemozoin was also found to activate the NLRP3 inflammasome during experimental malaria infections [109, 110].

P. falciparum genome is specially rich in AT-motifs. It was recently shown that these motifs have immunostimulatory properties and are able to induce type I IFNs. In fact, *Plasmodium* iRBCs triggered type I IFN production in macrophages [111]. DCs were not directly tested, but there is a very good possibility that they are activated in the same way.

GPI anchors are essential for *Plasmodium* survival and link different proteins to the parasite surface. *P. falciparum* merozoite-derived GPI anchors induced the production of proinflammatory cytokines and nitric oxide by macrophages [112, 113] mainly in a TLR2-dependent way but also, to a lesser extent, in a TLR4-dependent way [114], which requires CD36 cooperation [115]. *P. falciparum* GPIs' ability to activate DCs was evaluated more recently, and a study showed that TLR2-signaling was also important for DCs activation and induction of TNFα and IL-12 production. As observed for macrophages, CD36 also collaborated with TLR2 [116].

The participation of different TLRs was also evaluated in a *P. berghei* ANKA model of cerebral malaria. Results showed that mice deficient in TLR1, TLR2, TLR3, TLR4, TLR6, TLR7, or TLR9 and their adapter proteins MyD88, TIRAP, and TRIF were as susceptive to cerebral malaria as their wild-type counterparts [117, 118]. In contrast to these results, another group showed the contrary: cerebral malaria pathogenesis seems to be mediated by MyD88 signaling and its absence increases survival [119]. DCIR—a CLR that recognizes carbohydrates—deficient mice are resistant to cerebral malaria and present less $CD8^+$ T cell infiltration and inflammation in their brains when compared to wild-type mice [120].

In addition to PAMPs, *Plasmodium* infected erythrocytes were also shown to accumulate uric acid, a very potent endogenous danger-associated molecular pattern (DAMP). Uric acid precipitates accumulated within *P. falciparum* and *P. vivax* iRBC are released by cell rupture and induce the maturation of human dendritic cells *in vitro* [121]. Taken together, these results indicate that DCs sense and are able to respond to different *Plasmodium* PAMPs and also to DAMPs that are generated during infection.

5. Harnessing DCs for the Development of Vaccines against Malaria

DCs have also been studied as targets for the development of vaccines against malaria. The circumsporozoite protein (CSP)—the major sporozoite surface protein—from *P. yoelii* was fused to a monoclonal antibody capable of binding to a CLR—known as DEC205—expressed on the surface of the $CD8\alpha^+CD11b^-$ DCs subset. The administration of low doses of the αDEC205-CSP monoclonal antibody in the presence of a DCs maturation stimulus—αCD40+polyinosinic:polycytidylic acid, poly(I:C)—was able to induce IFNγ producing $CD4^+$ and $CD8^+$ T cells, besides specific anti-CSP antibodies [122]. The use of an anti-human DEC205 monoclonal antibody fused to the *P. falciparum* CSP together with poly(I:C) to immunize nonhuman primates elicited anti-CSP antibodies and also multifunctional $CD4^+$ T cell responses [123]. Another approach used to target the *P. yoelii* CSP to DCs was to fuse its sequence to the macrophage inflammatory protein 3α (MIP3α) that targets the CCR6 receptor present on the surface of immature DCs. Mice were immunized with a DNA plasmid encoding the MIP3α-CSP in the presence of Vaxfectin, and protection was obtained against the challenge [124]. These results indicate that DCs may be targeted with *Plasmodium* antigens in an attempt to induce potent immune responses and ultimately induce long-lasting protection.

6. Concluding Remarks

The effects of *Plasmodium* infection on dendritic cells are broad and normally result in DC activation and induction of potent T cell responses that also lead to B cell activation and antibody production. However, evidence has been gathered that malaria may also suppress DC function. The complexity of the parasite cycle and also the different DCs subsets contribute to increase the level of difficulty in understanding the outcome of all interactions. A multidisciplinary approach to elucidate the mechanisms involved in the activation of DCs by *Plasmodium* is necessary. If we understand how the parasite modulates DCs, it may be possible to manipulate this information to develop an effective vaccine against malaria.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

Acknowledgments

This review was supported by the São Paulo Research Foundation (FAPESP, 2007/08648-9 and 2013/11442-4), the Brazilian National Research Council (CNPq)/National Institutes of Science and Technology in Vaccines (INCTV, 15203*12), and BNP Paribas Bank. Kelly N. S. Amorim received a fellowship from CNPq, and Silvia B. Boscardin is supported by a fellowship from CNPq.

References

[1] M. C. Nussenzweig and R. M. Steinman, "Contribution of dendritic cells to stimulation of the murine syngeneic mixed leukocyte reaction," *Journal of Experimental Medicine*, vol. 151, no. 5, pp. 1196–1212, 1980.

[2] D. T. Avery, S. L. Kalled, J. I. Ellyard et al., "BAFF selectively enhances the survival of plasmablasts generated from human memory B cells," *Journal of Clinical Investigation*, vol. 112, no. 2, pp. 286–297, 2003.

[3] 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.

[4] 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," *The Journal of Immunology*, vol. 174, no. 2, pp. 727–734, 2005.

[5] C. Reis e Sousa, "Dendritic cells in a mature age," *Nature Reviews Immunology*, vol. 6, no. 6, pp. 476–483, 2006.

[6] T. Brocker, M. Riedinger, and K. Karjalainen, "Targeted expression of major histocompatibility complex (MHC) class II molecules demonstrates that dendritic cells can induce negative but not positive selection of thymocytes in vivo," *Journal of Experimental Medicine*, vol. 185, no. 3, pp. 541–550, 1997.

[7] R. M. Steinman and M. C. Nussenzweig, "Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 351–358, 2002.

[8] A. Iwasaki and R. Medzhitov, "Control of adaptive immunity by the innate immune system," *Nature Immunology*, vol. 16, no. 4, pp. 343–353, 2015.

[9] T. Kawai and S. Akira, "The role of pattern-recognition receptors in innate immunity: update on toll-like receptors," *Nature Immunology*, vol. 11, no. 5, pp. 373–384, 2010.

[10] J. C. Hoving, G. J. Wilson, and G. D. Brown, "Signalling C-type lectin receptors, microbial recognition and immunity," *Cellular Microbiology*, vol. 16, no. 2, pp. 185–194, 2014.

[11] V. A. K. Rathinam, S. K. Vanaja, and K. A. Fitzgerald, "Regulation of inflammasome signaling," *Nature Immunology*, vol. 13, no. 4, pp. 333–342, 2012.

[12] J. Wu and Z. J. Chen, "Innate immune sensing and signaling of cytosolic nucleic acids," *Annual Review of Immunology*, vol. 32, pp. 461–488, 2014.

[13] B. Lemaitre, E. Nicolas, L. Michaut, J.-M. Reichhart, and J. A. Hoffmann, "The dorsoventral regulatory gene cassette *spätzle/Toll/cactus* controls the potent antifungal response in Drosophila adults," *Cell*, vol. 86, no. 6, pp. 973–983, 1996.

[14] R. Luger, S. Valookaran, N. Knapp, C. Vizzardelli, A. M. Dohnal, and T. Felzmann, "Toll-like receptor 4 engagement drives differentiation of human and murine dendritic cells from a pro- into an anti-inflammatory mode," *PLoS ONE*, vol. 8, no. 2, Article ID e54879, pp. 1–13, 2013.

[15] M. Merad, P. Sathe, J. Helft, J. Miller, and A. Mortha, "The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting," *Annual Review of Immunology*, vol. 31, pp. 563–604, 2013.

[16] F. Ginhoux and M. Merad, "Ontogeny and homeostasis of Langerhans cells," *Immunology and Cell Biology*, vol. 88, no. 4, pp. 387–392, 2010.

[17] G. T. Belz, K. Shortman, M. J. Bevan, and W. R. Heath, "CD8α+ dendritic cells selectively present MHC class I-restricted noncytolytic viral and intracellular bacterial antigens in vivo," *Journal of Immunology*, vol. 175, no. 1, pp. 196–200, 2005.

[18] J. M. M. den Haan, S. M. Lehar, and M. J. Bevan, "CD8$^+$ but not CD8$^-$ dendritic cells cross-prime cytotoxic T cells in vivo," *Journal of Experimental Medicine*, vol. 192, no. 12, pp. 1685–1695, 2000.

[19] T.-P. Vu Manh, N. Bertho, A. Hosmalin, I. Schwartz-Cornil, and M. Dalod, "Investigating evolutionary conservation of dendritic cell subset identity and functions," *Frontiers in Immunology*, vol. 6, article 260, 2015.

[20] K. Liu and M. C. Nussenzweig, "Origin and development of dendritic cells," *Immunological Reviews*, vol. 234, no. 1, pp. 45–54, 2010.

[21] B. T. Edelson, K. C. Wumesh, R. Juang et al., "Peripheral CD103$^+$ dendritic cells form a unified subset developmentally related to CD8α^+ conventional dendritic cells," *The Journal of Experimental Medicine*, vol. 207, no. 4, pp. 823–836, 2010.

[22] H. Nakano, M. Yanagita, and M. D. Gunn, "CD11c$^+$B220$^+$Gr-1$^+$ cells in mouse lymph nodes and spleen display characteristics of plasmacytoid dendritic cells," *Journal of Experimental Medicine*, vol. 194, no. 8, pp. 1171–1178, 2001.

[23] C. Asselin-Paturel, A. Boonstra, M. Dalod et al., "Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology," *Nature Immunology*, vol. 2, no. 12, pp. 1144–1150, 2001.

[24] M. O'Keeffe, H. Hochrein, D. Vremec et al., "Mouse plasmacytoid cells: long-lived cells, heterogeneous in surface phenotype and function, that differentiate into CD8- dendritic cells only after microbial stimulus," *Journal of Experimental Medicine*, vol. 196, no. 10, pp. 1307–1319, 2002.

[25] G. Schlecht, S. Garcia, N. Escriou, A. A. Freitas, C. Leclerc, and G. Dadaglio, "Murine plasmacytoid dendritic cells induce effector/memory CD8$^+$ T-cell responses in vivo after viral stimulation," *Blood*, vol. 104, no. 6, pp. 1808–1815, 2004.

[26] J. A. Villadangos and L. Young, "Antigen-presentation properties of plasmacytoid dendritic cells," *Immunity*, vol. 29, no. 3, pp. 352–361, 2008.

[27] U. O'Doherty, M. Peng, S. Gezelter et al., "Human blood contains two subsets of dendritic cells, one immunologically mature and the other immature," *Immunology*, vol. 82, no. 3, pp. 487–493, 1994.

[28] A. Dzionek, A. Fuchs, P. Schmidt et al., "BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood," *The Journal of Immunology*, vol. 165, no. 11, pp. 6037–6046, 2000.

[29] B. Reizis, A. Bunin, H. S. Ghosh, K. L. Lewis, and V. Sisirak, "Plasmacytoid dendritic cells: recent progress and open questions," *Annual Review of Immunology*, vol. 29, pp. 163–183, 2011.

[30] F. O. Nestle, X.-G. Zheng, C. B. Thompson, L. A. Turka, and B. J. Nickoloff, "Characterization of dermal dendritic cells obtained from normal human skin reveals phenotypic and functionally distinctive subsets," *The Journal of Immunology*, vol. 151, no. 11, pp. 6535–6545, 1993.

[31] A. Boltjes and F. van Wijk, "Human dendritic cell functional specialization in steady-state and inflammation," *Frontiers in Immunology*, vol. 5, article 131, 2014.

[32] WHO, "World malaria report 2014," WHO Report, World Health Organization, 2014.

[33] R. Amino, S. Thiberge, B. Martin et al., "Quantitative imaging of *Plasmodium* transmission from mosquito to mammal," *Nature Medicine*, vol. 12, no. 2, pp. 220–224, 2006.

[34] L. M. Yamauchi Lucy, A. Coppi, G. Snounou, and P. Sinnis, "Plasmodium sporozoites trickle out of the injection site," *Cellular Microbiology*, vol. 9, no. 5, pp. 1215–1222, 2007.

[35] M. M. Mota, G. Pradel, J. P. Vanderberg et al., "Migration of Plasmodium sporozoites through cells before infection," *Science*, vol. 291, no. 5501, pp. 141–144, 2001.

[36] A. Sturm, R. Amino, C. van de Sand et al., "Manipulation of host hepatocytes by the malaria parasite for delivery into liver sinusoids," *Science*, vol. 313, no. 5791, pp. 1287–1290, 2006.

[37] A. Bartoloni and L. Zammarchi, "Clinical aspects of uncomplicated and severe malaria," *Mediterranean Journal of Hematology and Infectious Diseases*, vol. 4, no. 1, Article ID e2012026, 2012.

[38] W. R. J. Taylor, J. Hanson, G. D. H. Turner, N. J. White, and A. M. Dondorp, "Respiratory manifestations of malaria," *Chest*, vol. 142, no. 2, pp. 492–505, 2012.

[39] L. H. Miller, D. I. Baruch, K. Marsh, and O. K. Doumbo, "The pathogenic basis of malaria," *Nature*, vol. 415, no. 6872, pp. 673–679, 2002.

[40] J. W. Barnwell, A. S. Asch, R. L. Nachman, M. Yamaya, M. Aikawa, and P. Ingravallo, "A human 88-kD membrane glycoprotein (CD36) functions in vitro as a receptor for a cytoadherence ligand on *Plasmodium falciparum*-infected erythrocytes," *The Journal of Clinical Investigation*, vol. 84, no. 3, pp. 765–772, 1989.

[41] A. R. Berendt, D. L. Simmons, J. Tansey, C. I. Newbold, and K. Marsh, "Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*," *Nature*, vol. 341, no. 6237, pp. 57–59, 1989.

[42] J. A. Rowe, A. Claessens, R. A. Corrigan, and M. Arman, "Adhesion of *Plasmodium falciparum*-infected erythrocytes to human cells: molecular mechanisms and therapeutic implications," *Expert Reviews in Molecular Medicine*, vol. 11, article e16, 2009.

[43] P. C. Bull, B. S. Lowe, M. Kortok, C. S. Molyneux, C. I. Newbold, and K. Marsh, "Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria," *Nature Medicine*, vol. 4, no. 3, pp. 358–360, 1998.

[44] M. M. Stevenson and E. M. Riley, "Innate immunity to malaria," *Nature Reviews Immunology*, vol. 4, no. 3, pp. 169–180, 2004.

[45] J. K. Baird, T. R. Jones, E. W. Danudirgo et al., "Age-dependent acquired protection against *Plasmodium falciparum* in people having two years exposure to hyperendemic malaria," *The American Journal of Tropical Medicine and Hygiene*, vol. 45, no. 1, pp. 65–76, 1991.

[46] A. F. Egan, J. Morris, G. Barnish et al., "Clinical immunity to *Plasmodium falciparum* malaria is associated with serum antibodies to the 19-kDa C-terminal fragment of the merozoite surface antigen, PfMSP-1," *Journal of Infectious Diseases*, vol. 173, no. 3, pp. 765–769, 1996.

[47] D. Webster and A. V. S. Hill, "Progress with new malaria vaccines," *Bulletin of the World Health Organization*, vol. 81, no. 12, pp. 902–909, 2003.

[48] M. N. Wykes and M. F. Good, "What really happens to dendritic cells during malaria?" *Nature Reviews Microbiology*, vol. 6, no. 11, pp. 864–870, 2008.

[49] S.-I. Inoue, M. Niikura, S. Mineo, and F. Kobayashi, "Roles of IFN-γ and $\gamma\delta$ T cells in protective immunity against blood-stage malaria," *Frontiers in Immunology*, vol. 4, article 258, 2013.

[50] W. R. Weiss and C. G. Jiang, "Protective CD8+ T lymphocytes in primates immunized with malaria sporozoites," *PLoS ONE*, vol. 7, no. 2, Article ID e31247, 2012.

[51] W. R. Weiss, M. Sedegah, R. L. Beaudoin, L. H. Miller, and M. F. Good, "$CD8^+$ T cells (cytotoxic/suppressors) are required for protection in mice immunized with malaria sporozoites," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 85, no. 2, pp. 573–576, 1988.

[52] M. Tsuji, "A retrospective evaluation of the role of T cells in the development of malaria vaccine," *Experimental Parasitology*, vol. 126, no. 3, pp. 421–425, 2010.

[53] B. Wizel, R. A. Houghten, K. C. Parker et al., "Irradiated sporozoite vaccine induces HLA-B8-restricted cytotoxic T lymphocyte responses against two overlapping epitopes of the Plasmodium falciparum sporozoite surface protein 2," *Journal of Experimental Medicine*, vol. 182, no. 5, pp. 1435–1445, 1995.

[54] N. S. Butler, N. W. Schmidt, and J. T. Harty, "Differential effector pathways regulate memory CD8 T cell immunity against *Plasmodium berghei* versus *P. yoelii* sporozoites," *The Journal of Immunology*, vol. 184, no. 5, pp. 2528–2538, 2010.

[55] D. Perez-Mazliah and J. Langhorne, "CD4 T-cell subsets in malaria: Th1/Th2 revisited," *Frontiers in Immunology*, vol. 5, article 671, 2014.

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

[57] B. C. Urban, D. J. P. Ferguson, A. Pain et al., "Plasmodium falciparuminfected erythrocytes modulate the maturation of dendritic cells," *Nature*, vol. 400, no. 6739, pp. 73–77, 1999.

[58] S. R. Elliott, T. P. Spurck, J. M. Dodin et al., "Inhibition of dendritic cell maturation by malaria is dose dependent and does not require *Plasmodium falciparum* erythrocyte membrane protein 1," *Infection and Immunity*, vol. 75, no. 7, pp. 3621–3632, 2007.

[59] B. C. Urban, D. Cordery, M. J. Shafi et al., "The frequency of BDCA3-positive dendritic cells is increased in the peripheral circulation of Kenyan children with severe malaria," *Infection and Immunity*, vol. 74, no. 12, pp. 6700–6706, 2006.

[60] P. Guermonprez, J. Helft, C. Claser et al., "Inflammatory Flt3l is essential to mobilize dendritic cells and for T cell responses during Plasmodium infection," *Nature Medicine*, vol. 19, no. 6, pp. 730–738, 2013.

[61] B. C. Urban, M. J. Shafi, D. V. Cordery et al., "Frequencies of peripheral blood myeloid cells in healthy Kenyan children with alpha+ thalassemia and the sickle cell trait," *The American Journal of Tropical Medicine and Hygiene*, vol. 74, no. 4, pp. 578–584, 2006.

[62] R. M. Gonçalves, K. C. Salmazi, B. A. N. Santos et al., "CD4+ CD25+ Foxp3+ regulatory T cells, dendritic cells, and circulating cytokines in uncomplicated malaria: do different parasite species elicit similar host responses?" *Infection and Immunity*, vol. 78, no. 11, pp. 4763–4772, 2010.

[63] K. Jangpatarapongsa, P. Chootong, J. Sattabongkot et al., "*Plasmodium vivax* parasites alter the balance of myeloid and plasmacytoid dendritic cells and the induction of regulatory T cells," *European Journal of Immunology*, vol. 38, no. 10, pp. 2697–2705, 2008.

[64] A. Pinzon-Charry, T. Woodberry, V. Kienzle et al., "Apoptosis and dysfunction of blood dendritic cells in patients with falciparum and vivax malaria," *The Journal of Experimental Medicine*, vol. 210, no. 8, pp. 1635–1646, 2013.

[65] S. Kho, J. Marfurt, R. Noviyanti et al., "Preserved dendritic cell HLA-DR expression and reduced regulatory T cell activation in Asymptomatic Plasmodium falciparum and P. vivax infection," *Infection and Immunity*, vol. 83, no. 8, pp. 3224–3232, 2015.

[66] P. Vichchathorn, R. Jenwithisuk, S. Leelaudomlipi et al., "Induction of specific immune responses against the *Plasmodium vivax* liver-stage via in vitro activation by dendritic cells," *Parasitology International*, vol. 55, no. 3, pp. 187–193, 2006.

[67] O. Bruña-Romero and A. Rodriguez, "Dendritic cells can initiate protective immune responses against malaria," *Infection and Immunity*, vol. 69, no. 8, pp. 5173–5176, 2001.

[68] S. Jung, D. Unutmaz, P. Wong et al., "In vivo depletion of $CD11c^+$ dendritic cells abrogates priming of $CD8^+$ T cells by exogenous cell-associated antigens," *Immunity*, vol. 17, no. 2, pp. 211–220, 2002.

[69] S. Chakravarty, I. A. Cockburn, S. Kuk, M. G. Overstreet, J. B. Sacci, and F. Zavala, "CD8+ T lymphocytes protective against malaria liver stages are primed in skin-draining lymph nodes," *Nature Medicine*, vol. 13, no. 9, pp. 1035–1041, 2007.

[70] A. J. Radtke, W. Kastenmüller, D. A. Espinosa et al., "Lymph-node resident $CD8\alpha^+$ dendritic cells capture antigens from migratory malaria sporozoites and induce $CD8^+$ T cell responses," *PLoS Pathogens*, vol. 11, no. 2, Article ID e1004637, 2015.

[71] M. Plebanski, C. M. Hannan, S. Behboudi et al., "Direct processing and presentation of antigen from malaria sporozoites by professional antigen-presenting cells in the induction of CD8+ T-cell responses," *Immunology and Cell Biology*, vol. 83, no. 3, pp. 307–312, 2005.

[72] S. Behboudi, A. Moore, and A. V. S. Hill, "Splenic dendritic cell subsets prime and boost CD8 T cells and are involved in the generation of effector CD8 T cells," *Cellular Immunology*, vol. 228, no. 1, pp. 15–19, 2004.

[73] S. A. Murray, I. Mohar, J. L. Miller et al., "CD40 is required for protective immunity against liver stage *Plasmodium* infection," *Journal of Immunology*, vol. 194, no. 5, pp. 2268–2279, 2015.

[74] C. Ocaña-Morgner, M. M. Mota, and A. Rodriguez, "Malaria blood stage suppression of liver stage immunity by dendritic cells," *The Journal of Experimental Medicine*, vol. 197, no. 2, pp. 143–151, 2003.

[75] J. M. Orengo, K. A. Wong, C. Ocãa-Morgner, and A. Rodriguez, "A Plasmodium yoelii soluble factor inhibits the phenotypic maturation of dendritic cells," *Malaria Journal*, vol. 7, article 254, 2008.

[76] N. S. Wilson, G. M. N. Behrens, R. J. Lundie et al., "Systemic activation of dendritic cells by Toll-like receptor ligands or malaria infection impairs cross-presentation and antiviral immunity," *Nature Immunology*, vol. 7, no. 2, pp. 165–172, 2006.

[77] R. J. Lundie, T. F. de Koning-Ward, G. M. Davey et al., "Blood-stage Plasmodium infection induces $CD8^+$ T lymphocytes to parasite-expressed antigens, largely regulated by $CD8\alpha^+$ dendritic cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 38, pp. 14509–14514, 2008.

[78] J. A. Perry, A. Rush, R. J. Wilson, C. S. Olver, and A. C. Avery, "Dendritic cells from malaria-infected mice are fully functional APC," *The Journal of Immunology*, vol. 172, no. 1, pp. 475–482, 2004.

[79] J. Luyendyk, O. R. Olivas, L. A. Ginger, and A. C. Avery, "Antigen-presenting cell function during *Plasmodium yoelii* infection," *Infection and Immunity*, vol. 70, no. 6, pp. 2941–2949, 2002.

[80] E. Seixas, C. Cross, S. Quin, and J. Langhorne, "Direct activation of dendritic cells by the malaria parasite, *Plasmodium chabaudi chabaudi*," *European Journal of Immunology*, vol. 31, no. 10, pp. 2970–2978, 2001.

[81] A. L. Leisewitz, K. A. Rockett, B. Gumede, M. Jones, B. Urban, and D. P. Kwiatkowski, "Response of the splenic dendritic cell population to malaria infection," *Infection and Immunity*, vol. 72, no. 7, pp. 4233–4239, 2004.

[82] A.-M. Sponaas, E. T. Cadman, C. Voisine et al., "Malaria infection changes the ability of splenic dendritic cell populations to stimulate antigen-specific T cells," *Journal of Experimental Medicine*, vol. 203, no. 6, pp. 1427–1433, 2006.

[83] A.-M. Sponaas, N. Belyaev, M. Falck-Hansen, A. Potocnik, and J. Langhorne, "Transient deficiency of dendritic cells results in lack of a merozoite surface protein 1-specific CD4 T cell response during peak *Plasmodium chabaudi* blood-stage infection," *Infection and Immunity*, vol. 80, no. 12, pp. 4248–4256, 2012.

[84] J. Langhorne, F. M. Ndungu, A.-M. Sponaas, and K. Marsh, "Immunity to malaria: more questions than answers," *Nature Immunology*, vol. 9, no. 7, pp. 725–732, 2008.

[85] C. Voisine, B. Mastelic, A.-M. Sponaas, and J. Langhorne, "Classical CD11c+ dendritic cells, not plasmacytoid dendritic cells, induce T cell responses to Plasmodium chabaudi malaria," *International Journal for Parasitology*, vol. 40, no. 6, pp. 711–719, 2010.

[86] K. A. Wong and A. Rodriguez, "*Plasmodium* infection and endotoxic shock induce the expansion of regulatory dendritic cells," *Journal of Immunology*, vol. 180, no. 2, pp. 716–726, 2008.

[87] R. Ing, M. Segura, N. Thawani, M. Tam, and M. M. Stevenson, "Interaction of mouse dendritic cells and malaria-infected erythrocytes: Uptake, maturation, and antigen presentation," *Journal of Immunology*, vol. 176, no. 1, pp. 441–450, 2006.

[88] H. Borges da Silva, R. Fonseca, A. D. A. Cassado et al., "In vivo approaches reveal a key role for DCs in CD4+ T cell activation and parasite clearance during the acute phase of experimental blood-stage malaria," *PLoS Pathogens*, vol. 11, no. 2, Article ID e1004598, 2015.

[89] S. de Walick, F. H. Amante, K. A. McSweeney et al., "Cutting edge: conventional dendritic cells are the critical APC required for the induction of experimental cerebral malaria," *The Journal of Immunology*, vol. 178, no. 10, pp. 6033–6037, 2007.

[90] T. Tamura, K. Kimura, K. Yui, and S. Yoshida, "Reduction of conventional dendritic cells during Plasmodium infection is dependent on activation induced cell death by type I and II interferons," *Experimental Parasitology*, vol. 159, pp. 127–135, 2015.

[91] X. Q. Liu, K. J. Stacey, J. M. Horne-Debets et al., "Malaria infection alters the expression of B-cell activating factor resulting in diminished memory antibody responses and survival," *European Journal of Immunology*, vol. 42, no. 12, pp. 3291–3301, 2012.

[92] V. Ryg-Cornejo, C. Q. Nie, N. J. Bernard et al., "NK cells and conventional dendritic cells engage in reciprocal activation for the induction of inflammatory responses during *Plasmodium berghei* ANKA infection," *Immunobiology*, vol. 218, no. 2, pp. 263–271, 2013.

[93] S. Loharungsikul, M. Troye-Blomberg, P. Amoudruz et al., "Expression of Toll-like receptors on antigen-presenting cells in patients with falciparum malaria," *Acta Tropica*, vol. 105, no. 1, pp. 10–15, 2008.

[94] S. Pichyangkul, K. Yongvanitchit, U. Kum-Arb et al., "Malaria blood stage parasites activate human plasmacytoid dendritic cells and murine dendritic cells through a toll-like receptor 9-dependent pathway," *Journal of Immunology*, vol. 172, no. 8, pp. 4926–4933, 2004.

[95] B. S. Franklin, S. O. Rodrigues, L. R. Antonelli et al., "MyD88-dependent activation of dendritic cells and $CD4^+$ T lymphocytes mediates symptoms, but is not required for the immunological control of parasites during rodent malaria," *Microbes and Infection*, vol. 9, no. 7, pp. 881–890, 2007.

[96] R. T. Gazzinelli, P. Kalantari, K. A. Fitzgerald, and D. T. Golenbock, "Innate sensing of malaria parasites," *Nature Reviews Immunology*, vol. 14, no. 11, pp. 744–757, 2014.

[97] D. Jani, R. Nagarkatti, W. Beatty et al., "HDP—a novel heme detoxification protein from the malaria parasite," *PLoS Pathogens*, vol. 4, no. 4, Article ID e1000053, 2008.

[98] E. Schwarzer, M. Alessio, D. Ulliers, and P. Arese, "Phagocytosis of the malarial pigment, hemozoin, impairs expression of major histocompatibility complex class II antigen, CD54, and CD11c in human monocytes," *Infection and Immunity*, vol. 66, no. 4, pp. 1601–1606, 1998.

[99] O. A. Skorokhod, M. Alessio, B. Mordmüller, P. Arese, and E. Schwarzer, "Hemozoin (malarial pigment) inhibits differentiation and maturation of human monocyte-derived dendritic cells: a peroxisome proliferator-activated receptor-γ-mediated effect," *Journal of Immunology*, vol. 173, no. 6, pp. 4066–4074, 2004.

[100] O. Skorokhod, E. Schwarzer, T. Grune, and P. Arese, "Role of 4-hydroxynonenal in the hemozoin-mediated inhibition of differentiation of human monocytes to dendritic cells induced by GM-CSF/IL-4," *BioFactors*, vol. 24, no. 1–4, pp. 283–289, 2005.

[101] P. Giusti, B. C. Urban, G. Frascaroli et al., "*Plasmodium falciparum*-infected erythrocytes and β-hematin induce partial

maturation of human dendritic cells and increase their migratory ability in response to lymphoid chemokines," *Infection and Immunity*, vol. 79, no. 7, pp. 2727–2736, 2011.

[102] I. Bujila, E. Schwarzer, O. Skorokhod, J. M. Weidner, M. Troye-Blomberg, and A. Östlund Farrants, "Malaria-derived hemozoin exerts early modulatory effects on the phenotype and maturation of human dendritic cells," *Cellular Microbiology*, vol. 18, no. 3, pp. 413–423, 2016.

[103] O. R. Millington, C. Di Lorenzo, R. S. Phillips, P. Garside, and J. M. Brewer, "Suppression of adaptive immunity to heterologous antigens during *Plasmodium* infection through hemozoin-induced failure of dendritic cell function," *Journal of Biology*, vol. 5, no. 2, p. 5, 2006.

[104] O. R. Millington, V. B. Gibson, C. M. Rush et al., "Malaria impairs T cell clustering and immune priming despite normal signal 1 from dendritic cells," *PLoS Pathogens*, vol. 3, no. 10, pp. 1380–1387, 2007.

[105] C. Coban, K. J. Ishii, D. J. Sullivan, and N. Kumar, "Purified malaria pigment (hemozoin) enhances dendritic cell maturation and modulates the isotype of antibodies induced by a DNA vaccine," *Infection and Immunity*, vol. 70, no. 7, pp. 3939–3943, 2002.

[106] C. Coban, K. J. Ishii, T. Kawai et al., "Toll-like receptor 9 mediates innate immune activation by the malaria pigment hemozoin," *The Journal of Experimental Medicine*, vol. 201, no. 1, pp. 19–25, 2005.

[107] P. Parroche, F. N. Lauw, N. Goutagny et al., "Malaria hemozoin is immunologically inert but radically enhances innate responses by presenting malaria DNA to Toll-like receptor 9," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 6, pp. 1919–1924, 2007.

[108] X. Wu, N. M. Gowda, S. Kumar, and D. C. Gowda, "Protein-DNA complex is the exclusive malaria parasite component that activates dendritic cells and triggers innate immune responses," *Journal of Immunology*, vol. 184, no. 8, pp. 4338–4348, 2010.

[109] M. T. Shio, S. C. Eisenbarth, M. Savaria et al., "Malarial hemozoin activates the NLRP3 inflammasome through Lyn and Syk kinases," *PLoS Pathogens*, vol. 5, no. 8, Article ID e1000559, 2009.

[110] C. Dostert, G. Guarda, J. F. Romero et al., "Malarial hemozoin is a Nalp3 inflammasome activating danger signal," *PLoS ONE*, vol. 4, no. 8, Article ID e6510, 2009.

[111] S. Sharma, R. B. DeOliveira, P. Kalantari et al., "Innate immune recognition of an AT-rich stem-loop DNA motif in the Plasmodium falciparum genome," *Immunity*, vol. 35, no. 2, pp. 194–207, 2011.

[112] S. D. Tachado, P. Gerold, M. J. McConville et al., "Glycosylphosphatidylinositol toxin of *Plasmodium* induces nitric oxide synthase expression in macrophages and vascular endothelial cells by a protein tyrosine kinase-dependent and protein kinase C-dependent signaling pathway," *Journal of Immunology*, vol. 156, no. 5, pp. 1897–1907, 1996.

[113] J. Zhu, G. Krishnegowda, and D. C. Gowda, "Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of *Plasmodium falciparum*: the requirement of extracellular signal-regulated kinase, p38, c-Jun N-terminal kinase and NF-κB pathways for the expression of proinflammatory cytokines and nitric oxide," *The Journal of Biological Chemistry*, vol. 280, no. 9, pp. 8617–8627, 2005.

[114] G. Krishnegowda, A. M. Hajjar, J. Zhu et al., "Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of *Plasmodium falciparum*: cell signaling receptors, glycosylphosphatidylinositol (GPI) structural requirement, and regulation of GPI activity," *The Journal of Biological Chemistry*, vol. 280, no. 9, pp. 8606–8616, 2005.

[115] L. K. Erdman, G. Cosio, A. J. Helmers, D. C. Gowda, S. Grinstein, and K. C. Kain, "CD36 and TLR interactions in inflammation and phagocytosis: implications for malaria," *The Journal of Immunology*, vol. 183, no. 10, pp. 6452–6459, 2009.

[116] S. Kumar, N. M. Gowda, X. Wu, R. N. Gowda, and D. C. Gowda, "CD36 modulates proinflammatory cytokine responses to Plasmodium falciparum glycosylphosphatidylinositols and merozoites by dendritic cells," *Parasite Immunology*, vol. 34, no. 7, pp. 372–382, 2012.

[117] D. Togbe, L. Schofield, G. E. Grau et al., "Murine cerebral malaria development is independent of toll-like receptor signaling," *The American Journal of Pathology*, vol. 170, no. 5, pp. 1640–1648, 2007.

[118] B. Lepenies, J. P. Cramer, G. D. Burchard, H. Wagner, C. J. Kirschning, and T. Jacobs, "Induction of experimental cerebral malaria is independent of TLR2/4/9," *Medical Microbiology and Immunology*, vol. 197, no. 1, pp. 39–44, 2008.

[119] C. Coban, S. Uematsu, N. Arisue et al., "Pathological role of Toll-like receptor signaling in cerebral malaria," *International Immunology*, vol. 19, no. 1, pp. 67–79, 2007.

[120] M. Maglinao, R. Klopfleisch, P. H. Seeberger, and B. Lepenies, "The C-type lectin receptor DCIR is crucial for the development of experimental cerebral malaria," *Journal of Immunology*, vol. 191, no. 5, pp. 2551–2559, 2013.

[121] D. L. van de Hoef, I. Coppens, T. Holowka, C. Ben Mamoun, O. Branch, and A. Rodriguez, "*Plasmodium falciparum*-derived uric acid precipitates induce maturation of dendritic cells," *PLoS ONE*, vol. 8, no. 2, Article ID e55584, 2013.

[122] S. B. Boscardin, J. C. R. Hafalla, R. F. Masilamani et al., "Antigen targeting to dendritic cells elicits long-lived T cell help for antibody responses," *Journal of Experimental Medicine*, vol. 203, no. 3, pp. 599–606, 2006.

[123] K. Tewari, B. J. Flynn, S. B. Boscardin et al., "Poly(I:C) is an effective adjuvant for antibody and multi-functional CD4+ T cell responses to *Plasmodium falciparum* circumsporozoite protein (CSP) and αDEC-CSP in non human primates," *Vaccine*, vol. 28, no. 45, pp. 7256–7266, 2010.

[124] K. Luo, H. Zhang, F. Zavala, A. Biragyn, D. A. Espinosa, and R. B. Markham, "Fusion of antigen to a dendritic cell targeting chemokine combined with adjuvant yields a malaria dna vaccine with enhanced protective capabilities," *PLoS ONE*, vol. 9, no. 3, Article ID e90413, 2014.

[125] F. Ginhoux, K. Liu, J. Helft et al., "The origin and development of nonlymphoid tissue CD103$^+$ DCs," *The Journal of Experimental Medicine*, vol. 206, no. 13, pp. 3115–3130, 2009.

[126] E. Segura, J. Valladeau-Guilemond, M.-H. Donnadieu, X. Sastre-Garau, V. Soumelis, and S. Amigorena, "Characterization of resident and migratory dendritic cells in human lymph nodes," *Journal of Experimental Medicine*, vol. 209, no. 4, pp. 653–660, 2012.

Hindawi Publishing Corporation
Journal of Immunology Research
Volume 2016, Article ID 4753607, 10 pages
http://dx.doi.org/10.1155/2016/4753607

Review Article

Sensitivity of Dendritic Cells to Microenvironment Signals

Juliana Maria Motta and Vivian Mary Rumjanek

Laboratório de Imunologia Tumoral, Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, 21941-902 Rio de Janeiro, RJ, Brazil

Correspondence should be addressed to Juliana Maria Motta; jmotta@bioqmed.ufrj.br

Received 17 November 2015; Revised 3 February 2016; Accepted 6 March 2016

Academic Editor: Christine Trumpfheller

Dendritic cells are antigen-presenting cells capable of either activating the immune response or inducing and maintaining immune tolerance. They do this by integrating stimuli from the environment and changing their functional status as a result of plasticity. The modifications suffered by these cells have consequences in the way the organism may respond. In the present work two opposing situations known to affect dendritic cells are analyzed: tumor growth, leading to a microenvironment that favors the induction of a tolerogenic profile, and organ transplantation, which leads to a proinflammatory profile. Lessons learned from these situations may help to understand the mechanisms of modulation resulting not only from the above circumstances, but also from other pathologies.

1. Introduction

Although studies of the immune system mainly focus on its role in protecting against infections, this is only part of its function in helping to maintain the homeostasis of the organism. The tolerance and unresponsiveness to self-antigens, as well as the ability to terminate immune responses after pathogens control, are mechanisms carefully regulated and essential to keep and return to the steady-state. To be able to maintain the necessary equilibrium, the system must adapt to different challenges producing distinct and sometimes paradoxical responses.

Dendritic cells contribute to this purpose exhibiting a large spectrum of phenotypes and activities. The present review examines the role played by dendritic cells in two extremes and opposing situations (tumor microenvironment versus organ transplantation) where the plasticity of these cells is clearly observed and is directly related to their microenvironment.

2. Dendritic Cell Origin and Function

Dendritic cells are cells specialized in antigen presentation. These cells are capable of perceiving environment imbalances, capturing self and non-self-antigens, and processing and presenting them as peptides linked to the major histocompatibility complexes (MHC) to T lymphocytes. Dendritic cells are extremely sensitive to microenvironment signals and they scan the organism, especially the sites where there is more probability of antigen entrance. In other words, dendritic cells efficiently instruct the adaptive immune system in response to peripheral cues, as discussed by Merad et al. [1].

Evidence suggests that dendritic cells are originated from both myeloid and lymphoid hematopoietic progenitors. The cytokine Flt3 ligand (Flt3L) was shown to be necessary for dendritic cell development in the bone marrow of both human and mice. Furthermore, this cytokine plays a role later in murine and human lymphoid organs. Deficiency of its receptor (Flt3) is associated with these cells depletion in mice [2–5]. *In vivo*, it was demonstrated that Flt3L administration can drive and expand dendritic cell generation along both the lymphoid and myeloid developmental pathways from $Flt3^+$ progenitor cells [3].

In humans, dendritic cells represent only 0.1–0.5% of mononuclear cells present in peripheral blood [6]. Due to the low concentration and difficulty in obtaining these cells, the work with human dendritic cells was limited for years. However, obtaining human dendritic cells, *in vitro*, from bone marrow precursors or monocyte-induced differentiation has made it possible to study the biology of these cells [7, 8].

Granulocyte-macrophage colony-stimulating factor (GM-CSF) combined with tumor necrosis factor-alpha (TNF-α) is efficient in differentiating bone marrow $CD34^+$ cells into dendritic cells [7, 9], while the best mixture to differentiate monocytes into dendritic cells is interleukin-4 (IL-4) plus GM-CSF [8]. In this context, GM-CSF was characterized by inducing the expansion of progenitor cells, as well as promoting their differentiation and survival. TNF-α induces differentiation and stimulates the proliferation of hematopoietic $CD34^+$ cells. IL-4, in turn, inhibits the formation of macrophage colonies [10]. Monocyte-derived dendritic cells can be activated with CD40L or TNF-α, since the cells have already been differentiated [8].

A number of alterations regarding surface proteins are observed during differentiation. Monocytes gradually lose CD14 expression yet start to express molecules of CD1 family [8, 11]. When activated, they increase the expression of costimulatory molecules, such as CD80, CD86, and CD40, and express high levels of CD83 and MHC class II [12, 13]. The expression of chemokine receptors also changes during their maturation. Immature dendritic cells express receptors to inflammatory chemokines, such as CCR1, CCR2, CCR5, CCR6, and CXCR1, which facilitate their targeting of the site of inflammation. During activation, CCR6 expression is downregulated, while CCR7 and CXCR4 are upregulated, allowing the movement of cells toward lymph nodes [14–16]. In parallel to these changes, cells modify their activity during development. Immature dendritic cells are strongly capable of endocytosis of potential antigens through different mechanisms involving a number of pattern recognition receptors, whereas activated cells have this ability diminished, albeit displaying increased allostimulatory activity [17, 18].

3. Dendritic Cell Plasticity and Subtypes

Dendritic cells are heterogeneous and dynamic cells. Dendritic cells were first characterized by Steinman and Cohn, who isolated them from the spleen of animals and described their remarkable motility and usual conformational change. They also highlighted the fact that, in contrast to B lymphocytes, dendritic cells were unable to increase significantly their numbers in an immune response [19–21]. Therefore, dendritic cell plasticity was always suspected although only recently this has become evident.

These cells can be classified according to their functional development: dendritic cells that are resident in peripheral nonlymphoid tissues and able to recognize and process antigens are called immature. After capturing antigens, dendritic cells migrate to secondary lymphoid organs and acquire the ability to activate lymphocytes. At this point in development, they are classified as mature or activated dendritic cells [22]. Despite some divergence in the literature, these cells are also classified in accordance to their phenotypic differences. Two groups have been proposed: conventional or classical dendritic cells, a group characterized by an integrin expression (CD11c) and in which Langerhans cells and interstitial dendritic cells are included; plasmacytoid dendritic cells, which are producers of interferon (IFN) type I (α and β), therefore, mainly involved in viral infection responses [23–26]. Nevertheless, there are controversies especially regarding plasmacytoid dendritic cells, due to the fact that they present morphological and phenotypical characteristics of both lymphocytes and classical dendritic cells. A comparative study between human lymphoid and myeloid progenitor cells showed that both can give rise to classical and plasmacytoid cells [25]. In contrast to classical dendritic cells, human plasmacytoid cells do not express CD11c, express low levels of costimulatory molecules and MHC class II, and express a common marker of B lymphocytes, B220. They also differ in the pattern of toll-like receptors (TLRs) expressed, with plasmacytoid cells expressing more TLR 7 and 9 [1, 26].

Independently from the classification, dendritic cells possess the capacity of switching between tolerogenic and effector/cytotoxic phenotypes. Studies in mice characterized immature dendritic cells as cells prone to develop tolerogenic responses while activated dendritic cells were more efficient at promoting effective responses by T cells. This conclusion was based on the fact that dendritic cells resident in peripheral tissues are generally immature and, under homeostatic situations, they induce anergy or T regulatory cell development [27, 28]. The generalization that involves immature dendritic cells as promoters of tolerance versus activated dendritic cells as inducers of effective immune response was accepted for a long time; however, certain stimuli that promote dendritic cell activation are also capable of inducing tolerance, like microbial-derived products [29]. Another example is the treatment of activated dendritic cells with IFN-γ which promotes the expression of indoleamine 2,3-dioxygenase (IDO) leading these cells to acquire tolerogenic properties that could be reverted by the inhibition of IDO [30]. Therefore, dendritic cell activities are not dependent on the activation state and they represent a complex group with multiple functional intermediates as opposed to immature and activated cells [31, 32].

Dendritic cell tolerance to self-antigens and to resident nonpathological microorganisms is as essential as the capacity of being immunogenic when a pathogen is present; thus, their ability to switch from these two phenotypes must be finely regulated.

4. Dendritic Cells in the Tumor Microenvironment

In the tumor microenvironment the tolerogenic pathway is increased in relation to the effector pathway. Moreover, this microenvironment is generally suppressive to immune cells, which means that immune functions are often prevented, consequently leading to unresponsiveness. Many cell types are affected by tumor cells contact and their various released products. For instance, $CD8^+$ T lymphocytes have their cytotoxicity ability compromised [33], NK cells are impaired [34], and macrophages acquire a M2-like phenotype [35, 36].

Dendritic cells are also strongly susceptible to tumor products that may induce important alterations. Analyzing dendritic cell differentiation from human $CD34^+$ progenitor cells, the vascular endothelial growth factor (VEGF) was the first tumor-derived protein described as a suppressor of this process [37]. Moreover, it was shown that serine

proteases secreted by prostate tumor cells and gangliosides from various tumors inhibited dendritic cell generation in a manner similar to the development (from $CD34^+$ cells) in both, humans and mice [38, 39].

Using a different model, monocyte-induced differentiation toward dendritic cells, Menetrier-Caux and collaborators showed that this process was also modulated by tumor products [40]. IL-6 and macrophage colony-stimulating factor (M-CSF) produced by tumors and macrophages present in the tumor microenvironment suppress dendritic cell differentiation, whereas they stimulate macrophage differentiation through the increase of M-CSF receptor expression in monocytes [40].

As discussed by Zou, in 2005, the concentration of cytokines that favor dendritic cell development and function, like GM-CSF, IL-4, IL-12, and IFN-γ, is very low, while factors that suppress dendritic cells, such as IL-6, IL-10, prostaglandin E2 (PGE2), VEGF, and transforming growth factor-beta (TGF-β), are found in higher levels [34]. In summary, the imbalance of cytokines found in tumor microenvironment does not favor dendritic cell development.

Clinical studies revealed a correlation between the number of dendritic cells inside the tumor and the survival of patients [41]. The presence of a consistent number of $CD1a^+$ cells in the tumor microenvironment is associated with a better prognosis [42, 43]. Moreover, Joo and coworkers showed that monocyte-derived $CD1a^+$ dendritic cells, during activation process, induce apoptosis and cell cycle arrest in breast cancer cells via secretion of soluble products [44].

Despite the importance of dendritic cells during an antitumoral response, there is clear evidence pointing to the fact that dendritic cells are strongly suppressed by factors present in the tumor microenvironment. Both blood (systemically) and tumor microenvironment (locally) dendritic cells seem to be functionally compromised, which means that tumor products may affect cells in distant sites and possibly undifferentiated cells in the bone marrow [45]. The presence of activated dendritic cells is rare in tumor areas and it has been demonstrated in ovarian, breast, prostate, and some renal carcinomas. All steps of dendritic cell development, migration, and activity may present significant defects [46–49].

Some workers have tried to reveal the mechanisms involved in tumor-related inhibition of dendritic cells. Among them, Sombroek and collaborators demonstrated that primary tumors (renal carcinoma, cervical cancer, breast cancer and melanoma) negatively modulate the development and activity of dendritic cells through factors regulated by cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) [50]. In our hands, investigating a plausible regulation of COX-2 in developing dendritic cells, it was possible to determine that this enzyme was upregulated when cells were under the stimulation of leukemic cell products (Figure 1). Here, once more, we could detect an impairment of dendritic cell development by tumor-derived products.

In another work, Kiertscher et al. showed that monocytes $CD14^+$ respond to products present in tumor cell cultures by increasing the expression of antigen-presenting cells surface receptors and increasing the translocation of

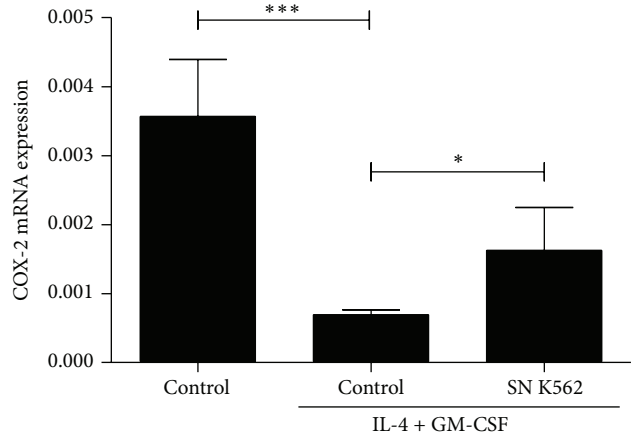


Figure 1: mRNA expression of COX-2 by monocytes differentiated with leukemic cell products. Mononuclear cells were obtained after gradient density from buffy coats of healthy individuals. After 2 h of adhesion, lymphocytes were removed and monocyte cultures were stimulated with IL-4 and GM-CSF (50 ng/mL) to induce dendritic cell differentiation. K562 supernatants (SN K562) were obtained after 3 days of cell culture in RPMI plus 10% of fetal bovine serum (FBS) followed by filtering (0.22 μm). 10% of SN K562 was used since the beginning of monocyte culture until the end (5 days). Afterward, total mRNA was collected using trizol, retrotranscription was performed, and, finally, qPCR was done using COX-2 specific primers. The graph shows mean ± SEM of expression of COX-2 mRNA. $^*p \leq 0.05$; $^{***}p \leq 0.001$. $n = 5$.

nuclear factors [51]. However, despite having activated dendritic cells characteristics, these cells lose their ability to secrete IL-12, do not acquire allostimulatory capacity, and rapidly undergo apoptosis [51]. Furthermore, it was shown that cervical adenocarcinoma cells affect the generation of dendritic cells which become incapable of producing IL-12. This has been attributed to the production of IL-10 by tumor cells and consequently to a less expression of CD40 by dendritic cells [52]. Blocking VEGF was the approach used by Osada and coworkers in a study with lung, breast, and colon carcinoma patient cells. They were able to show that anti-VEGF treatment increases cell ability of activating and promoting lymphocyte proliferation [53].

Most studies have been performed with products obtained from solid tumors. In 2010, our group showed that soluble products released by leukemic cells inhibited dendritic cell differentiation through the induction of IL-1β and this effect could be partially reversed when IL-1β was neutralized in culture [54]. Because we knew the importance of IL-1β to tumor progression and metastasis [55], we decided to study the effect of this cytokine along with tumor products on dendritic cell differentiation. Stimuli resulting from tumor products or from IL-1β produced different results regarding the appearance of macrophage markers in these cells, with leukemic cell products increasing CD68 and CD16 expression, a feature not observed with IL-1β. On the other hand, similarities involving a suppressive phenotype were demonstrated with both kinds of stimuli [56].

In addition to preventing dendritic cell generation and function and stimulating tolerogenic pathways, tumors can

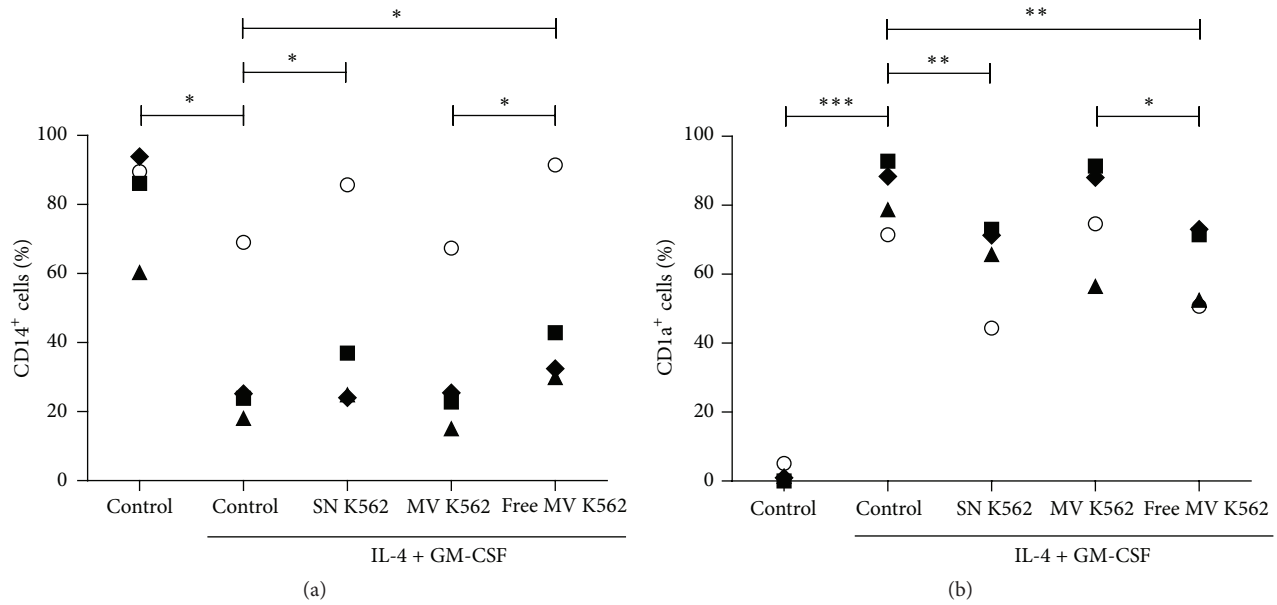


Figure 2: Leukemic cell microvesicles influence on dendritic cell differentiation. Mononuclear cells were obtained after gradient density from buffy coats of healthy individuals. After 2 h of adhesion, lymphocytes were removed and monocyte cultures were stimulated with IL-4 and GM-CSF (50 ng/mL) to induce dendritic cell differentiation. K562 supernatants (SN K562) were obtained after 3 days of cell culture in RPMI plus 10% of fetal bovine serum (FBS) followed by filtering (0.22 μm). Part of the tumor supernatant was centrifuged twice at 100000 g for 1 h to purify microvesicles. After this process, two supernatants were obtained: K562 supernatant only with microvesicles resuspended in the same original volume of medium (MV K562) and K562 supernatant with all the other products except microvesicles (free MV K562). Supernatants were added (10% of final volume) at the beginning of monocyte culture and remained until the end (5 days). Afterward, cells were stained with anti-CD14 FITC and anti-CD1a PE and data were acquired in a FACS Calibur. Graphs show the percentage of $CD14^+$ (a) and $CD1a^+$ (b) cells. Each individual analyzed in different conditions is represented by one symbol. $^*p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$. $n = 4$.

induce specific cell phenotypes, such as regulatory dendritic cells and myeloid-derived suppressor cells (MDSCs). Tumor products may induce immature dendritic cells conversion into regulatory dendritic cells, which promote T regulatory cell activation and produce TGF-β [32]. In contrast, MDSCs were described as a more undifferentiated population and were characterized in humans by the expression of CD33 [57]. Recent studies showed that the appearance of both phenotypes is directly related to tumor progression and metastasis establishment [32].

Tumors not only represent a pathological condition that is associated with cellular changes, but also represent an organized system of communication between different cells. Diverse and numerous cell types communicate and collaborate in order to establish a favorable environment for the development of cancer. One of the forms of communication used by normal cells, but amplified in neoplastic cells, is the secretion of microvesicles [58]. Microparticles or microvesicles are plasma membrane fragments released by almost all cell types when subjected to stress conditions, including apoptosis. For a long time they were considered only cellular debris. However, more recently it has been shown *in vitro* that microvesicles may reflect cell activation and, *in vivo*, tissue degeneration in various pathophysiological conditions [59]. These structures can vary widely in size (the diameter varies within 0.1–1 μm) and also with regard to their composition [60].

The association of microvesicles with tumors is deduced by the fact that they are secreted in larger amounts by tumor cells and it explains why they circulate at higher levels in the peripheral blood of cancer patients [61–63]. *In vitro* studies demonstrated that microvesicles accumulate in tumor cell cultures stimulated or not [64]. Some tumor microvesicles inhibitory effects on immune cells have been described. Baj-Krzyworzeka and collaborators showed that tumor microvesicles interact with monocytes by changing their phenotype [65]. Furthermore, effects like the blockage of proliferation, cytotoxic activity, and the induction of apoptosis in lymphocytes have been shown [66–68]. Suppression of MHC class II molecules expression by murine macrophages was also described [69]. These data suggest that microvesicles derived from tumors may have immunosuppressive characteristics. In our experiments, using leukemic cell products during monocyte differentiation into dendritic cells, we failed to associate inhibition of this process with microvesicles (Figure 2). Here, the comparison performed was between microvesicles-free tumor supernatants and supernatants in which only microvesicles were present. It was observed that the effect on blocking the decrease of CD14 and CD1a appearance was restricted to microvesicles-free supernatant, thus eliminating the role of these tumor microvesicles at least on the CD14 and CD1a modulation (Figure 2).

Furthermore, experiments were performed to verify whether tumor cell products responsible for the modulation

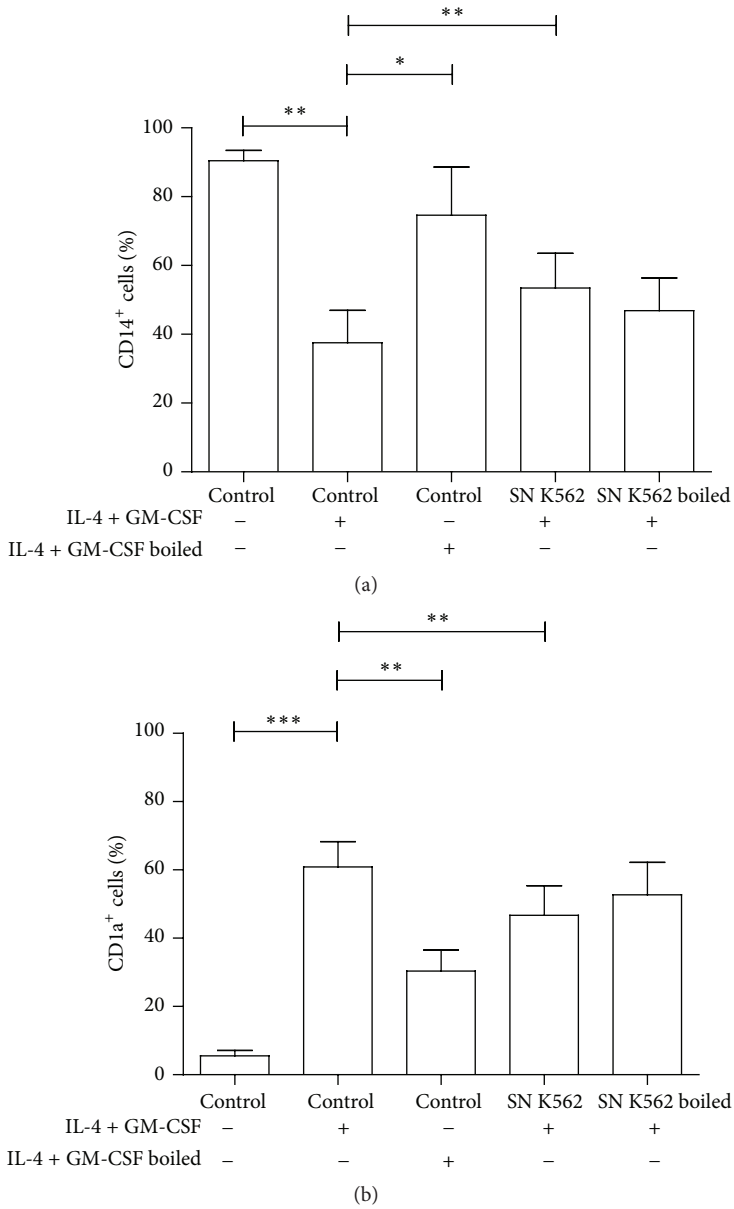


Figure 3: Leukemic cell-derived proteins effect on monocytes differentiating into dendritic cells. Mononuclear cells were obtained after gradient density from buffy coats of healthy individuals. After 2 h of adhesion, lymphocytes were removed and monocyte cultures were stimulated with IL-4 and GM-CSF (50 ng/mL) to induce dendritic cell differentiation. K562 supernatants (SN K562) were obtained after 3 days of cell culture in RPMI plus 10% of fetal bovine serum (FBS) followed by filtering (0.22 μm). Part of tumor supernatant and part of IL-4 and GM-CSF were boiled for 10 minutes at 100°C in order to denature proteins. After cooling, some cells were incubated with these supernatants or cytokines at the same concentration described above for 5 days. Anti-CD14 FITC and anti-CD1a PE were used to stain the cells and data were assessed by a FACS Calibur. Graphs show mean ± SEM of the percentage of $CD14^+$ (a) and $CD1a^+$ (b) cells. $^*p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$. $n \geq 5$.

obtained were of a proteic nature. To that end, the tumor supernatant was compared to a supernatant that was boiled at 100°C. In some individuals analyzed, a small reversion of the effect on CD14 and CD1a expression was observed when supernatants were heated, indicating the involvement of any product with a proteic nature. However, residual effects still persisted (Figure 3). Moreover, this was not seen in all the experiments carried out when IL-4 and GM-CSF were heated (used as control). They were still able to induce partial differentiation (Figure 3). Therefore, further investigation is required to better identify the nature of leukemic cell products responsible for dendritic cell generation impairment.

5. Dendritic Cell Role in Allograft Rejection

Transplantation is a therapy used for the majority of bone marrow-derived cancers and for many metabolic disorders or failure of diverse organs. Serious complications may occur

in transplantation. Bone marrow transplanted patients might suffer from graft-versus-host disease. On the other hand, solid organ transplanted patients might suffer from graft failure. In both situations the risk of infection is a serious problem. Dealing with these issues, it is of great relevance for the understanding of immune mechanisms able to control effective and tolerogenic responses. Contrary to what has been done for immunotherapy against cancer, proposals to avoid transplant rejection are geared towards increasing the ratio tolerance/effective response.

In a model of renal transplantation, an increased number of monocyte-derived dendritic cells in mature stage were found. Moreover this dendritic cell subset was more frequent than the classical dendritic cells that are usually present in major numbers in the kidney. Therefore, a consistent infiltration of monocytes was proposed by Zuidwijk et al. [70]. Another subset of dendritic cells seems to be related to kidney graft rejection. Although plasmacytoid dendritic cells have been described as cells closely associated with tolerance, studies with renal transplantation acute rejection showed that the presence of this subset is not rare. Thus, it could be correlated to inflammation as these plasmacytoid dendritic cells are important producers of IFN [71, 72].

Graft-resident dendritic cells seem to have a protective activity whereas infiltrating dendritic cells might act as proinflammatory cells. This idea was reinforced by experiments with animals in which the progressive infiltration of dendritic cells and $CD4^+$ T lymphocytes increased effector responses against the graft in a kidney model of transplantation [73].

Interestingly, liver transplantation is better accepted in patients and its grafting may be able to enhance the acceptance of other organs, like heart and skin allografts. Therefore, it was proposed that the liver microenvironment favors immune tolerance not only locally, but also systematically [74]. Most dendritic cells that reside in the liver display a typical immature phenotype with low expression of costimulatory molecules. Furthermore, dendritic cell-mediated tolerance in liver can be explained by the expression of programmed death ligand 1 (PD-L1) by these cells as well as cytotoxic T lymphocyte antigen 4 (CTLA-4) that was found to be highly expressed by T lymphocytes in the organ [75, 76]. Even though liver dendritic cells may produce IL-12, the IL-10 level is increased in this organ compared to others and, in part, it may explain the tolerogenic behavior of liver-resident dendritic cells [74, 77].

Certain subsets of dendritic cells have been related to heart graft rejection. A role for $CD11c^+$ dendritic cells in heart graft rejection has been demonstrated by Oberhuber et al. [78]. Making use of a murine model, they showed that intragraft $CD11c^+$ cells enhance $CD4^+$ and $CD8^+$ T cells responses and the production of cytokines, such as IL-17A. Conversely, depletion of this population of dendritic cells or blocking of IL-17A is able to delay the rejection of transplanted heart [78].

The generation of regulatory dendritic cells in this circumstance could be interesting. Murine regulatory dendritic cells that lack the expression of costimulatory proteins were induced *in vitro* and injected in mice 7 days before heart transplantation. Results demonstrated longer graft survival even in mice that were not treated with immunosuppressive drugs [79, 80].

One of the most studied types of transplantation is the skin graft due to the high index of rejection. Dermal dendritic cells and Langerhans cells (present in epidermis) are the main subsets of dendritic cells in normal skin. In skin transplantation, both populations of dendritic cells migrate out of the graft in the direction of draining lymph nodes where they present donor antigens to T recipient cells. In this context, T cells from the recipient may recognize antigens from the donor dendritic cells (direct allorecognition) and also donor antigens presented by recipient dendritic cell (indirect allorecognition) [81, 82]. A third way of T cell activation is the recognition of donor MHC previous transferred to recipient dendritic cells (semidirect recognition) [83]. Thus, the activation of T cells occurs in a very efficient way in skin transplantation and it explains why this kind of graft is frequently rejected.

Solid organs transplantations require immunosuppressive therapies in order to ensure graft acceptance. However, bone marrow transplantation is unique because in this case the recipient immune system is destroyed and replaced by the donor system. Therefore, the new immune system is capable of rejecting not the graft but all the other organs. Immunosuppressive therapies are also required for this kind of transplantation, even though for different reasons [84].

The induction of peripheral tolerance is the main goal to avoid graft rejections and manipulating dendritic cells to acquire a tolerogenic activity is one of the mechanisms to reach this objective. Carreño and collaborators suggested the blockage of NF-κB as a way to induce tolerogenic characteristics in dendritic cells [85]. Recently, some immunosuppressive agents, which have been used to treat autoimmune disease and to avoid allograft rejection, were described as inductors of tolerance in dendritic cells [86].

6. Final Considerations

The plasticity of dendritic cells is evident and it is essential to keep the homeostasis of normal individuals. However, due to the capacity of these cells to easily switch phenotypes according to microenvironmental signals, this plasticity might be harmful when disorders such as cancer occur or when an allotransplantation is required (Figure 4). Nevertheless, lessons learned on how these situations may affect dendritic cells might help to understand how to handle the modulation connected to modifications in the microenvironment resulting from diverse pathologies. Here we discussed two paradoxical situations in which the balance of tolerance/effective immune response was considered especially looking at dendritic cell phenotype and function.

Competing Interests

The authors declare that they have no competing interests.

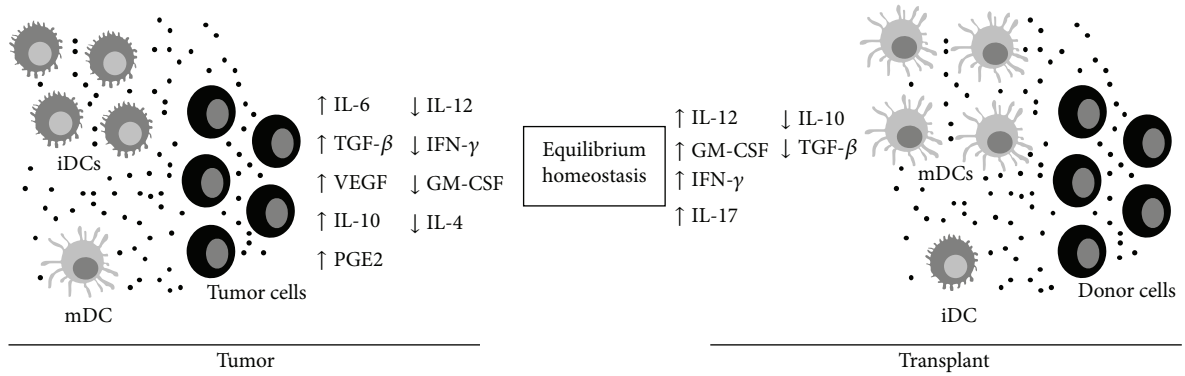


Figure 4: Schematic illustration showing the differences between tumor and transplants microenvironments. Immature dendritic cells (iDCs) are present in higher number in tumor microenvironments, while activated dendritic cells (mDCs) can be more easily found in transplanted organs. In both cases, tumor cells or donor cells and dendritic cells are in constant communication through cytokines and other factors secretion (represented by small black circles). In the tumor microenvironment, factors that are generally suppressive to dendritic cells are present in big amounts. On the other hand, in transplants, proinflammatory cytokines which exacerbate dendritic cells function are increased.

Acknowledgments

This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and INCT-Controle do Câncer. Buffy coats from healthy volunteers were gently provided by Serviço de Hemoterapia, Hospital Universitário Clementino Fraga Filho. The authors would like to thank Dr. Robson de Queiroz Monteiro and his students for helping them with some experiments and also Dr. Franklin David Rumjanek for critical review of the paper.

References

[1] M. Merad, P. Sathe, J. Helft, J. Miller, and A. Mortha, "The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting," *Annual Review of Immunology*, vol. 31, pp. 563–604, 2013.

[2] A. D'Amico and L. Wu, "The early progenitors of mouse dendritic cells and plasmacytoid predendritic cells are within the bone marrow hemopoietic precursors expressing Flt3," *The Journal of Experimental Medicine*, vol. 198, no. 2, pp. 293–303, 2003.

[3] H. Karsunky, M. Merad, A. Cozzio, I. L. Weissman, and M. G. Manz, "Flt3 ligand regulates dendritic cell development from Flt3+ lymphoid and myeloid-committed progenitors to Flt3+ dendritic cells in vivo," *Journal of Experimental Medicine*, vol. 198, no. 2, pp. 305–313, 2003.

[4] N. Onai, A. Obata-Onai, R. Tussiwand, A. Lanzavecchia, and M. G. Manz, "Activation of the Flt3 signal transduction cascade rescues and enhances type I interferon-producing and dendritic cell development," *Journal of Experimental Medicine*, vol. 203, no. 1, pp. 227–238, 2006.

[5] C. Waskow, K. Liu, G. Darrasse-Jèze et al., "The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues," *Nature Immunology*, vol. 9, no. 6, pp. 676–683, 2008.

[6] W. C. Van Voorhis, L. S. Hair, R. M. Steinman, and G. Kaplan, "Human dendritic cells. Enrichment and characterization from peripheral blood," *The Journal of Experimental Medicine*, vol. 155, no. 4, pp. 1172–1187, 1982.

[7] C. D. L. Reid, A. Stackpoole, A. Meager, and J. Tikerpae, "Interactions of tumor necrosis factor with granulocyte-macrophage colony-stimulating factor and other cytokines in the regulation of dendritic cell growth in vitro from early bipotent CD34+ progenitors in human bone marrow," *The Journal of Immunology*, vol. 149, no. 8, pp. 2681–2688, 1992.

[8] F. Sallusto and A. Lanzavecchia, "Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α," *The Journal of Experimental Medicine*, vol. 179, no. 4, pp. 1109–1118, 1994.

[9] C. Caux, C. Dezutter-Dambuyant, D. Schmit, and J. Banchereau, "GM-CSF and TNF-α cooperate in the generation of dendritic Langerhans cells," *Nature*, vol. 360, no. 6401, pp. 258–261, 1992.

[10] G. M. Zou and Y. K. Tam, "Cytokines in the generation and maturation of dendritic cells: recent advances," *European Cytokine Network*, vol. 13, no. 2, pp. 186–199, 2002.

[11] N. Romani, S. Gruner, D. Brang et al., "Proliferating dendritic cell progenitors in human blood," *Journal of Experimental Medicine*, vol. 180, no. 1, pp. 83–93, 1994.

[12] B. Obermaier, M. Dauer, J. Herten, K. Schad, S. Endres, and A. Eigler, "Development of a new protocol for 2-day generation of mature dendritic cells from human monocytes," *Biological Procedures Online*, vol. 5, no. 1, pp. 197–203, 2003.

[13] L.-J. Zhou and T. F. Tedder, "Human blood dendritic cells selectively express CD83, a member of the immunoglobulin superfamily," *Journal of Immunology*, vol. 154, no. 8, pp. 3821–3835, 1995.

[14] M.-C. Dieu, B. Vanbervliet, A. Vicari et al., "Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites," *Journal of Experimental Medicine*, vol. 188, no. 2, pp. 373–386, 1998.

[15] S. Sozzani, P. Allavena, G. D'Amico et al., "Cutting edge: differential regulation of chemokine receptors during dendritic cell maturation: a model for their trafficking properties," *The Journal of Immunology*, vol. 161, no. 3, pp. 1083–1086, 1998.

[16] F. Sallusto, A. Lanzavecchia, and C. R. MacKay, "Chemokines and chemokine receptors in T-cell priming and Th1/Th2-mediated responses," *Immunology Today*, vol. 19, no. 12, pp. 568–574, 1998.

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

[18] J. Banchereau, F. Briere, C. Caux et al., "Immunobiology of dendritic cells," *Annual Review of Immunology*, vol. 18, pp. 767–811, 2000.

[19] R. M. Steinman and Z. A. Cohn, "Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution," *The Journal of Experimental Medicine*, vol. 137, no. 5, pp. 1142–1162, 1973.

[20] R. M. Steinman and Z. A. Cohn, "Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties in vitro," *The Journal of Experimental Medicine*, vol. 139, no. 2, pp. 380–397, 1974.

[21] R. M. Steinman, D. S. Lustig, and Z. A. Cohn, "Identification of a novel cell type in peripheral lymphoid organs of mice. III. Functional properties in vivo," *The Journal of Experimental Medicine*, vol. 139, no. 6, pp. 1431–1445, 1974.

[22] B. J. C. Quah and H. C. O'Neill, "Maturation of function in dendritic cells for tolerance and immunity," *Journal of Cellular and Molecular Medicine*, vol. 9, no. 3, pp. 643–654, 2005.

[23] M. Arpinati, G. Chirumbolo, B. Urbini, G. Perrone, D. Rondelli, and C. Anasetti, "Role of plasmacytoid dendritic cells in immunity and tolerance after allogeneic hematopoietic stem cell transplantation," *Transplant Immunology*, vol. 11, no. 3-4, pp. 345–356, 2003.

[24] B. Reizis, "Regulation of plasmacytoid dendritic cell development," *Current Opinion in Immunology*, vol. 22, no. 2, pp. 206–211, 2010.

[25] F. Ishikawa, H. Niiro, T. Iino et al., "The developmental program of human dendritic cells is operated independently of conventional myeloid and lymphoid pathways," *Blood*, vol. 110, no. 10, pp. 3591–3600, 2007.

[26] B. Reizis, A. Bunin, H. S. Ghosh, K. L. Lewis, and V. Sisirak, "Plasmacytoid dendritic cells: recent progress and open questions," *Annual Review of Immunology*, vol. 29, pp. 163–183, 2011.

[27] R. M. Steinman, D. Hawiger, and M. C. Nussenzweig, "Tolerogenic dendritic cells," *Annual Review of Immunology*, vol. 21, pp. 685–711, 2003.

[28] R. M. Steinman and M. C. Nussenzweig, "Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 351–358, 2002.

[29] B. Pulendran, H. Tang, and S. Manicassamy, "Programming dendritic cells to induce T(H)2 and tolerogenic responses," *Nature Immunology*, vol. 11, no. 8, pp. 647–655, 2010.

[30] D. H. Munn, M. D. Sharma, J. R. Lee et al., "Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase," *Science*, vol. 297, no. 5588, pp. 1867–1870, 2002.

[31] M. B. Lutz and G. Schuler, "Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity?" *Trends in Immunology*, vol. 23, no. 9, pp. 445–449, 2002.

[32] Y. Ma, G. V. Shurin, D. W. Gutkin, and M. R. Shurin, "Tumor associated regulatory dendritic cells," *Seminars in Cancer Biology*, vol. 22, no. 4, pp. 298–306, 2012.

[33] I. Algarra, A. García-Lora, T. Cabrera, F. Ruiz-Cabello, and F. Garrido, "The selection of tumor variants with altered expression of classical and nonclassical MHC class I molecules: Implications for tumor immune escape," *Cancer Immunology, Immunotherapy*, vol. 53, no. 10, pp. 904–910, 2004.

[34] W. Zou, "Immunosuppressive networks in the tumour environment and their therapeutic relevance," *Nature Reviews Cancer*, vol. 5, no. 4, pp. 263–274, 2005.

[35] A. Mantovani, S. Sozzani, M. Locati, P. Allavena, and A. Sica, "Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes," *Trends in Immunology*, vol. 23, no. 11, pp. 549–555, 2002.

[36] M. Baay, A. Brouwer, P. Pauwels, M. Peeters, and F. Lardon, "Tumor cells and tumor-associated macrophages: secreted proteins as potential targets for therapy," *Clinical and Developmental Immunology*, vol. 2011, Article ID 565187, 12 pages, 2011.

[37] D. I. Gabrilovich, H. L. Chen, K. R. Girgis et al., "Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells," *Nature Medicine*, vol. 2, no. 10, pp. 1096–1103, 1996.

[38] M. Aalamian, I. L. Tourkova, G. S. Chatta et al., "Inhibition of dendropoiesis by tumor derived and purified prostate specific antigen," *Journal of Urology*, vol. 170, no. 5, pp. 2026–2030, 2003.

[39] G. V. Shurin, M. R. Shurin, S. Bykovskaia, J. Shogan, M. T. Lotze, and E. M. Barksdale, "Neuroblastoma-derived gangliosides inhibit dendritic cell generation and function," *Cancer Research*, vol. 61, no. 1, pp. 363–369, 2001.

[40] C. Menetrier-Caux, G. Montmain, M. C. Dieu et al., "Inhibition of the differentiation of dendritic cells from $CD34^+$ progenitors by tumor cells: role of interleukin-6 and macrophage colony-stimulating factor," *Blood*, vol. 92, no. 12, pp. 4778–4791, 1998.

[41] R. M. Dallal, P. Christakos, K. Lee, S. Egawa, Y.-I. Son, and M. T. Lotze, "Paucity of dendritic cells in pancreatic cancer," *Surgery*, vol. 131, no. 2, pp. 135–138, 2002.

[42] B. J. Coventry and J. Morton, "CD1a-positive infiltrating-dendritic cell density and 5-year survival from human breast cancer," *British Journal of Cancer*, vol. 89, no. 3, pp. 533–538, 2003.

[43] E. E. Hillenbrand, A. M. Neville, and B. J. Coventry, "Immunohistochemical localization of CD1a-positive putative dendritic cells in human breast tumours," *British Journal of Cancer*, vol. 79, no. 5-6, pp. 940–944, 1999.

[44] H.-G. Joo, T. P. Fleming, Y. Tanaka et al., "Human dendritic cells induce tumor-specific apoptosis by soluble factors," *International Journal of Cancer*, vol. 102, no. 1, pp. 20–28, 2002.

[45] M. D. Roth, B. J. Gitlitz, S. M. Kiertscher et al., "Granulocyte macrophage colony-stimulating factor and interleukin 4 enhance the number and antigen-presenting activity of circulating CD14+ and CD83+ cells in cancer patients," *Cancer Research*, vol. 60, no. 7, pp. 1934–1941, 2000.

[46] W. Zoul, V. Machelon, A. Coulomb-L'Hermin et al., "Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells," *Nature Medicine*, vol. 7, no. 12, pp. 1339–1346, 2001.

[47] D. Bell, P. Chomarat, D. Broyles et al., "In breast carcinoma tissue, immature dendritic cells reside within the tumor, whereas mature dendritic cells are located in peritumoral areas," *Journal of Experimental Medicine*, vol. 190, no. 10, pp. 1417–1426, 1999.

[48] A. Troy, P. Davidson, C. Atkinson, and D. Hart, "Phenotypic characterisation of the dendritic cell infiltrate in prostate cancer," *Journal of Urology*, vol. 160, no. 1, pp. 214–219, 1998.

[49] A. J. Troy, K. L. Summers, P. J. T. Davidson, C. H. Atkinson, and D. N. J. Hart, "Minimal recruitment and activation of dendritic cells within renal cell carcinoma," *Clinical Cancer Research*, vol. 4, no. 3, pp. 585–593, 1998.

[50] C. C. Sombroek, A. G. M. Stam, A. J. Masterson et al., "Prostanoids play a major role in the primary tumor-induced inhibition of dendritic cell differentiation," *The Journal of Immunology*, vol. 168, no. 9, pp. 4333–4343, 2002.

[51] S. M. Kiertscher, J. Luo, S. M. Dubinett, and M. D. Roth, "Tumors promote altered maturation and early apoptosis of monocyte-derived dendritic cells," *Journal of Immunology*, vol. 164, no. 3, pp. 1269–1276, 2000.

[52] M. R. Shurin, Z. R. Yurkovetsky, I. L. Tourkova, L. Balkir, and G. V. Shurin, "Inhibition of CD40 expression and CD40-mediated dendritic cell function by tumor-derived IL-10," *International Journal of Cancer*, vol. 101, no. 1, pp. 61–68, 2002.

[53] T. Osada, G. Chong, R. Tansik et al., "The effect of anti-VEGF therapy on immature myeloid cell and dendritic cells in cancer patients," *Cancer Immunology, Immunotherapy*, vol. 57, no. 8, pp. 1115–1124, 2008.

[54] J. M. Motta, C. R. Nascimento, and V. M. Rumjanek, "Leukemic cell products down-regulate human dendritic cell differentiation," *Cancer Immunology, Immunotherapy*, vol. 59, no. 11, pp. 1645–1653, 2010.

[55] R. N. Apte, S. Dotan, M. Elkabets et al., "The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumor-host interactions," *Cancer and Metastasis Reviews*, vol. 25, no. 3, pp. 387–408, 2006.

[56] J. M. Motta, A. Sperandio, M. T. L. Castelo-Branco, and V. M. Rumjanek, "Induction of suppressive phenotype in monocyte-derived dendritic cells by leukemic cell products and IL-1β," *Human Immunology*, vol. 75, no. 7, pp. 641–649, 2014.

[57] M. G. Lechner, C. Megiel, S. M. Russell et al., "Functional characterization of human $Cd33^+$ And $Cd11b^+$ myeloid-derived suppressor cell subsets induced from peripheral blood mononuclear cells co-cultured with a diverse set of human tumor cell lines," *Journal of Translational Medicine*, vol. 9, article no. 90, 2011.

[58] D. Castellana, F. Toti, and J.-M. Freyssinet, "Membrane microvesicles: macromessengers in cancer disease and progression," *Thrombosis research*, vol. 125, supplement 2, pp. S84–S88, 2010.

[59] J.-M. Freyssinet, "Cellular microparticles: what are they bad or good for?" *Journal of Thrombosis and Haemostasis*, vol. 1, no. 7, pp. 1655–1662, 2003.

[60] J. Ratajczak, K. Miekus, M. Kucia et al., "Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery," *Leukemia*, vol. 20, no. 5, pp. 847–856, 2006.

[61] A. Ginestra, M. D. La Placa, F. Saladino, D. Cassarà, H. Nagase, and M. L. Vittorelli, "The amount and proteolytic content of vesicles shed by human cancer cell lines correlates with their in vitro invasiveness," *Anticancer Research*, vol. 18, no. 5, pp. 3433–3437, 1998.

[62] K. Shedden, X. T. Xie, P. Chandaroy, Y. T. Chang, and G. R. Rosania, "Expulsion of small molecules in vesicles shed by cancer cells: association with gene expression and chemosensitivity profiles," *Cancer Research*, vol. 63, no. 15, pp. 4331–4337, 2003.

[63] H. K. Kim, K. S. Song, Y. S. Park et al., "Elevated levels of circulating platelet microparticles, VEGF, IL-6 and RANTES in patients with gastric cancer: possible role of a metastasis predictor," *European Journal of Cancer*, vol. 39, no. 2, pp. 184–191, 2003.

[64] L. G. Lima, R. Chammas, R. Q. Monteiro, M. E. C. Moreira, and M. A. Barcinski, "Tumor-derived microvesicles modulate the establishment of metastatic melanoma in a phosphatidylserine-dependent manner," *Cancer Letters*, vol. 283, no. 2, pp. 168–175, 2009.

[65] M. Baj-Krzyworzeka, R. Szatanek, K. Węglarczyk et al., "Tumour-derived microvesicles carry several surface determinants and mRNA of tumour cells and transfer some of these determinants to monocytes," *Cancer Immunology, Immunotherapy*, vol. 55, no. 7, pp. 808–818, 2006.

[66] D. D. Taylor, C. Gerçel-Taylor, K. S. Lyons, J. Stanson, and T. L. Whiteside, "T-cell apoptosis and suppression of T-cell receptor/CD3-zeta by Fas ligand containing membrane vesicles shed from ovarian tumors," *Clinical Cancer Research*, vol. 9, no. 14, pp. 5113–5119, 2003.

[67] G. Andreola, L. Rivoltini, C. Castelli et al., "Induction of lymphocyte apoptosis by tumor cell secretion of FasL-bearing microvesicles," *The Journal of Experimental Medicine*, vol. 195, no. 10, pp. 1303–1316, 2002.

[68] J. W. Kim, E. Wieckowski, D. D. Taylor, T. E. Reichert, S. Watkins, and T. L. Whiteside, "Fas ligand-positive membranous vesicles isolated from sera of patients with oral cancer induce apoptosis of activated T lymphocytes," *Clinical Cancer Research*, vol. 11, no. 3, pp. 1010–1020, 2005.

[69] D. D. Poutsiaka, E. W. Schroder, D. D. Taylor, E. M. Levy, and P. H. Black, "Membrane vesicles shed by murine melanoma cells selectively inhibit the expression of Ia antigen by macrophages," *Journal of Immunology*, vol. 134, no. 1, pp. 138–144, 1985.

[70] K. Zuidwijk, J. W. De Fijter, M. J. K. Mallat et al., "Increased influx of myeloid dendritic cells during acute rejection is associated with interstitial fibrosis and tubular atrophy and predicts poor outcome," *Kidney International*, vol. 81, no. 1, pp. 64–75, 2012.

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

[72] E. Martín-Gayo, E. Sierra-Filardi, A. L. Corbí, and M. L. Toribio, "Plasmacytoid dendritic cells resident in human thymus drive natural Treg cell development," *Blood*, vol. 115, no. 26, pp. 5366–5375, 2010.

[73] K. S. Ozaki, S. Kimura, M. A. Nalesnik et al., "The loss of renal dendritic cells and activation of host adaptive immunity are long-term effects of ischemia/reperfusion injury following syngeneic kidney transplantation," *Kidney International*, vol. 81, no. 10, pp. 1015–1025, 2012.

[74] A. Carambia and J. Herkel, "CD4 T cells in hepatic immune tolerance," *Journal of Autoimmunity*, vol. 34, no. 1, pp. 23–28, 2010.

[75] V. Racanelli and B. Rehermann, "The liver as an immunological organ," *Hepatology*, vol. 43, no. 2, pp. S54–S62, 2006.

[76] T. L. Sumpter, M. Abe, D. Tokita, and A. W. Thomson, "Dendritic cells, the liver, and transplantation," *Hepatology*, vol. 46, no. 6, pp. 2021–2031, 2007.

[77] G. Tiegs and A. W. Lohse, "Immune tolerance: what is unique about the liver," *Journal of Autoimmunity*, vol. 34, no. 1, pp. 1–6, 2010.

[78] R. Oberhuber, T. Heinbokel, H. R. Cetina Biefer et al., "$CD11c^+$ dendritic cells accelerate the rejection of older cardiac transplants via interleukin-17A," *Circulation*, vol. 132, no. 2, pp. 122–131, 2015.

[79] L. Lu, D. Mccaslin, T. E. Starzl, and A. W. Thomson, "Bone marrow-derived dendritic cell progenitors (NLDC 145^+, MHC

class II^{+}, B7-1^{dim}, B7-2^{-}) induce alloantigen-specific hyporesponsiveness in murine T lymphocytes," *Transplantation*, vol. 60, no. 12, pp. 1539–1545, 1995.

[80] F. Fu, Y. Li, S. Qian et al., "Costimulatory molecule-deficient dendritic cell progenitors (MHC class II+, CD80(dim), CD86-) prolong cardiac allograft survival in nonimmunosuppressed recipients," *Transplantation*, vol. 62, no. 5, pp. 659–665, 1996.

[81] C. P. Larsen, J. M. Austyn, and P. J. Morris, "The role of graft-derived dendritic leukocytes in the rejection of vascularized organ allografts. Recent findings on the migration and function of dendritic leukocytes after transplantation," *Annals of Surgery*, vol. 212, no. 3, pp. 308–315, 1990.

[82] G. Benichou, P. A. Takizawa, C. A. Olson, M. McMillan, and E. E. Sercarz, "Donor major histocompatibility complex (MHC) peptides are presented by recipient MHC molecules during graft rejection," *The Journal of Experimental Medicine*, vol. 175, no. 1, pp. 305–308, 1992.

[83] O. B. Herrera, D. Golshayan, R. Tibbott et al., "A novel pathway of alloantigen presentation by dendritic cells," *The Journal of Immunology*, vol. 173, no. 8, pp. 4828–4837, 2004.

[84] J. L. Zakrzewski, M. R. M. Van Den Brink, and J. A. Hubbell, "Overcoming immunological barriers in regenerative medicine," *Nature Biotechnology*, vol. 32, no. 8, pp. 786–794, 2014.

[85] L. J. Carreño, C. A. Riedel, and A. M. Kalergis, "Induction of tolerogenic dendritic cells by NF-κB blockade and Fcγ receptor modulation," *Methods in Molecular Biology*, vol. 677, pp. 339–353, 2011.

[86] L. Adorini, N. Giarratana, and G. Penna, "Pharmacological induction of tolerogenic dendritic cells and regulatory T cells," *Seminars in Immunology*, vol. 16, no. 2, pp. 127–134, 2004.

Hindawi Publishing Corporation
Journal of Immunology Research
Volume 2016, Article ID 5946807, 13 pages
http://dx.doi.org/10.1155/2016/5946807

Review Article

Dendritic Cells and Their Role in Cardiovascular Diseases: A View on Human Studies

Maja-Theresa Dieterlen,[1] Katja John,[1] Hermann Reichenspurner,[2] Friedrich W. Mohr,[1] and Markus J. Barten[2]

[1]*Department of Cardiac Surgery, Heart Center, Helios Clinic, University Hospital Leipzig, 04289 Leipzig, Germany*
[2]*Department of Cardiovascular Surgery, University Heart Center Hamburg, 20246 Hamburg, Germany*

Correspondence should be addressed to Maja-Theresa Dieterlen; mdieterlen@web.de

Received 23 November 2015; Revised 22 January 2016; Accepted 22 February 2016

Academic Editor: Silvia B. Boscardin

The antigen-presenting dendritic cells (DCs) are key to the immunological response, with different functions ascribed ranging from cellular immune activation to induction of tolerance. Such immunological responses are involved in the pathophysiological mechanisms of cardiovascular diseases, with DCs shown to play a role in atherosclerosis, hypertension, and heart failure and most notably following heart transplantation. A better understanding of the interplay between the immune system and cardiovascular diseases will therefore be critical for developing novel therapeutic treatments as well as innovative monitoring tools for disease progression. As such, the present review will provide an overview of DCs involvement in the pathophysiology of cardiovascular diseases and how targeting these cells may have beneficial effects for the prognosis of patients.

1. Introduction

Dendritic cells (DCs) and their antigen-presenting properties possess a central role in the immune system, with many diseases associated with a heightened immune response. Among these, cardiovascular diseases (CVDs) represent the most frequent causes of death worldwide, with an estimated 17.3 million deaths per year [1]. While most research has been done in the field of heart transplantation, more recently, DCs were also shown to be involved in numerous other CVDs. This present review, therefore, will discuss the underlying role of DCs in the immunological mechanisms that underpin the development and progression of CVDs. In contrast to previous reviews, the present essay will focus exclusively on human rather than animal studies, as the different phenotypical and functional DC subsets between groups [2] can often lead to misleading conclusions.

2. Immunological Mechanisms

DCs patrol the blood and peripheral tissue to detect foreign and pathogenic antigens. According to their hematopoietic origin, DCs can be divided into myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). Additionally, numerous surface markers have been shown to be useful for the identification of multiple DC subsets. For example, blood dendritic cell antigens (BDCA1–BDCA4) can be used to discriminate among DC types [3, 4], while further markers can also classify DCs such as S100 (expressed in mature and immature DCs), CD1a (expressed mainly in immature DCs), and CD83 (expressed mainly in mature DCs). Furthermore, specialized "cardiac" DCs have been found in the human heart [5–7], with immunohistochemical and morphological analyses revealing a subtype of DC expressing human leukocyte antigen- (HLA-) DR (but not S100, CD1a, CD21, CD23, and CD35 [5]). This different surface marker profile compared to ordinary DCs therefore led to the hypothesis that cardiac DCs change their characteristics depending on their location either in the blood vessels or in the heart [5].

The detection of foreign or pathogenic antigens (pathogen-associated molecular patterns, PAMPs) as well as tissue damage and inflammation (damage-associated molecular patterns, DAMPs) leads to phagocytosis of antigens by DCs, the latter then expressing the maturation marker CD83

and class I and class II major histocompatibility complexes (MHC). Mature, antigen-presenting DCs migrate to secondary lymphoid tissue where they present antigens to T cells [8]. While the homing of immature DCs is regulated by chemokine receptors (CCRs) on the DC surface, homing of mature DCs to regional lymph nodes is mediated by CCR7 and CXC-chemokine receptor type 4 (CXCR4) [9–11]. In the secondary lymphoid tissue, T cells are activated by receiving three signals from mature DCs as follows: (1) mature DCs present an antigen bound to a MHC molecule; (2) T cells then require costimulators such as CD80 or CD86; and (3) cytokines such as IL-12, IL-23, and IL-27 finally are secreted [12]. T cell activation leads to proliferation and differentiation of T cells into regulatory T cells (T_{regs}), T helper cells (T_H), or killer T cells. In general, DCs can activate all types of effector T cells and regulate activation and regulation of immune responses, which are both involved in disease patterns of CVDs.

3. DCs in Atherosclerosis and Aortocoronary Bypass

Atherosclerosis is the dominant cause of CVDs leading to myocardial infarction (MI), heart failure (HF), or stroke [13]. The investigation of the underlying pathophysiological mechanisms shows that immune cells such as T cells, monocytes, and DCs invade the vascular wall stimulated by oxidized LDL, TNF-α, and hypoxia [14, 15], which are often found in atherosclerotic lesions where they produce proinflammatory cytokines [16]. Both PAMPs and DAMPs can activate DCs [17] which subsequently mature, while further atherogenic factors in the vascular wall such as oxidized low-density lipoprotein (LDL) cholesterol [18], advanced glycation end products (AGE) [19], nicotine [20], insulin [21], and angiotensin II [22] also have the capacity to induce the maturation of DCs. Mature DCs activate T cells and initiate the upregulation of DC licensing factors such as CD40L [17]. These processes contribute to chronic vascular inflammation and form the basis for vascular obliteration.

Several reports on immunohistochemical analyses of carotid specimens raised the suggestion that DCs contribute to plaque destabilization, possibly through activation of T cells [23–26]. Yilmaz and colleagues analysed mDCs in atherosclerotic plaques in 44 carotid specimens and reported that advanced plaques had higher numbers of mDCs and a higher percentage of mature mDCs than initial lesions [23]. These observations were also confirmed in another patient study ($n = 29$), when unstable compared to stable plaques showed a 1.6-fold increase in both fascin$^+$ mDCs and S100$^+$ DCs, while a 5.9-fold increase of mature CD83$^+$ mDCs was observed [26]. However, while these observations could not be detected in pDCs [26], alternative DC markers such as BDCA-1 and BDCA-2 have revealed that mDCs and pDCs are indeed recruited to advanced plaques [27].

In addition, the research work of the Weyand laboratory further showed that 53% of the 30 carotid endarterectomy samples contained CD123$^+$ pDCs, but also CD11c$^+$ DC-Sign$^+$ fascin$^+$ mDCs, which are both located either in the shoulder region of the plaque or at the plaque base. The mDC/pDC ratio in the plaques was 2.7, and further characterization of the pDCs revealed that these cells were the main source of interferon-α. The number of pDCs as well as interferon-α transcript concentrations strongly correlated with plaque instability in the tissue samples [24, 25]. Further research on the cytokine and chemokine expression in atherosclerotic plaques from coronary artery disease (CAD) patients revealed that the T cell cytokines, interferon-γ and TNF-α, as well as DC chemokines, CCL19 and CCL21, are increased in patients with ischemic symptoms compared to asymptomatic patients [28].

Beyond DC tissue analyses, circulating DCs hold significant value in patients suffering from atherosclerosis, as supported by CAD patients having increased number of DCs in the atherosclerotic vascular wall concomitant with decreased levels of circulating DCs in the blood [29–33]. While Yilmaz et al. reported a reduction of circulating mDCs, pDCs, and total DCs in patients with advanced CAD [31] and mDCs in patients with angina pectoris and MI [29], Van Vré et al. found that absolute and relative numbers of circulating pDCs were lowered in 18 CAD patients compared to age- and sex-matched controls [30]. Interestingly, the same group further reported an inverse correlation between mDCs and IL-6 and C-reactive protein, suggesting that these cytokines may be involved in their regulation [32]. Other factors may also play a role, such as IL-23 and IL-23R [34] and tyrosine kinase 3 ligand (Flt3L) [33], which have also been correlated to pDC levels.

A large clinical study provided further evidence of a strong association between the roles of DCs in CAD, with 290 patients classified as "early CAD," "moderate CAD," "advanced CAD," and "CAD excluded" by coronary angiogram. In summary, the study demonstrated an inverse correlation between the CAD score and mDCs, pDCs, and total DCs, which were also independent predictors of CAD [31]. Yet noteworthy, patients undergoing percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) had lower total DCs and both DC subsets (mDCs and pDCs) compared to no intervention, suggesting that DC levels might be predictive of the targeted therapy after coronary angiogram [31].

Increased levels of DCs are also present in stenotic vein coronary bypass grafts [35]. In comparison to atherosclerosis of coronary arteries, the degeneration of vein grafts proceeds more rapidly [36] and finally leads to graft failure. In general, atherosclerosis develops in almost 50% of all vein grafts within ten years [37]. Cherian et al. investigated the presence of DCs in vein grafts and found DCs positive for S100 and CD1a in the vascular walls of these vessel grafts [35]. A further study on stenotic vein coronary bypass grafts demonstrated that DCs positive for the costimulatory molecule CD40 were clustered within the intima as well as in the media and adventitia [38]. In accordance with the results on human coronary bypass grafts, it has been demonstrated that T cells were accumulated in the vascular wall of saphenous vein grafts [39]. These data indicate that interactions between DCs and T cells are involved in the rapid development of atherosclerosis and degeneration of vein grafts, which finally promote eventual graft failure.

4. Hypertension and DCs

Hypertension is one of the most common chronic diseases, which promotes atherosclerosis and represents a major risk factor for CVD-related death [40]. A number of studies have suggested that immunological mechanisms, especially the inflammatory responses, are involved in hypertension [41–44]. Macrophages and lymphocytes infiltrate the interstitium in angiotensin II-induced hypertension [41], where T cells increase in the adventitia of blood vessels and secrete cytokines such as tumor necrosis factor-α (TNF-α) and IL-17 as well as NADPH oxidase [43, 45], which then lead to elevated blood pressure. This suggestion is reinforced by observations that immunosuppression causes reduced hypertension-induced end-organ damage while immunodeficiency reduces hypertension [42, 44].

While one research arm is related to the investigation of T cell variations in hypertension, another should represent T cell activating cells, specifically DCs. Indeed, Abbas et al. showed that hypertension activates DCs [34] and also further confirmed that reactive oxygen species (ROS) produced by DCs through phagocyte oxidase caused lipid oxidation, which resulted in accumulation of proteins that were oxidatively modified by highly reactive γ-ketoaldehydes (isoketals). The isoketal-modified proteins behave like DAMPs and activate DCs, which start to express IL-6, IL-1β, and IL-23 and the costimulators CD80 and CD86. The isoketal-pulsed DCs induced T cell proliferation, particularly of $CD8^+$ and IFN-γ and IL-17A, with the latter shown to elevate blood pressure [46]. Thus, one key mechanism of hypertension could be related to an autoimmune component [47], which is supported by evidence that isoketal proteins were traceable in mDCs in hypertensive compared to normotensive controls. Unfortunately, *in vivo* studies of DCs in hypertension are not well investigated, with only one study on DCs in hypertensive patients [47].

5. DCs and Heart Failure

Inflammation and immune responses are processes that can lead to HF such as myocarditis and cardiomyopathy. Myocarditis is an inflammatory heart disease that can be initiated by infectious viruses (e.g., Coxsackie B virus, Parvovirus) or the parasite *Trypanosoma cruzi* [48]. These pathogens infect cardiomyocytes, which cause direct tissue injury but also initiate immune responses against pathogenic antigens that lead to further tissue damage. In addition, myocarditis has an autoimmune component driven by molecular "mimicry" between microbial and myocardial self-antigens [49]. Molecular mimicry means that specific structures of certain pathogens imitate defined cardiac self-antigens [50]. Subsequently, the T cell response against such microorganisms includes the expansion of self-reactive T cells with the potential to attack the myocardium [51]. For example, structural proteins from *Chlamydia* strains mimic myosin and induce myocarditis after immunizing mice with homologous *Chlamydia* peptides [52].

A histological study of cardiac samples from autopsied patients with myocarditis ($n = 22$) and from an age- und sex-matched control group ($n = 20$) provided evidence that HLA-DR-positive cardiac DCs proliferate in the acute phase of myocarditis [5]. Cardiac DCs showed typical morphology of DCs with large cellular processes and were in close contact with myocytes, suggesting that cardiac DCs exert a destructive effect on myocytes. This hypothesis is supported by the fact that necrotic lesions were surrounded by infiltrating HLA-DR-positive cells, with dendritic-forming mononuclear cells also in the immediate environment. Similarly, polymorphonuclear giant cells, cardiac DCs, and T cells have also been detected in active inflammatory lesions in chronic patients [5].

MI causes progressive remodeling of myocardial tissue and impairs contractile function, with eventual progression to HF [53]. Immunological and inflammatory processes play an important role in cardiac remodeling after MI [54], with DCs playing a central role in mediating immunological effects following MI by their role in the development of autoimmunological processes and maintenance of peripheral tolerance. For example, MI is characterized by the uptake and presentation of myocardial peptides by DCs resulting in T cell activation. The infiltration of mature activated $CD11c^+$ $CD11b^+$ DCs into the infarcted heart, as well as an association between mature DCs and the deterioration of left ventricle remodeling, has been demonstrated in experimental MI [7, 55]. Furthermore, DCs act as a potent immunoprotective regulator during the post-MI healing process via DC control of the monocyte/macrophage homeostasis [54], with it being demonstrated early after MI where DCs activate not only regulatory T cells (T_{regs}), which are purported to prevent tissue-destructive autoimmunity after cardiac injury [56], but also other T cell subsets such as $CD4^+$ T cells [56] and $CD4^+$ T cells [57].

The effects on DC populations measured in human tissue are dependent on the type of MI [58, 59]. For example, a study on infarct tissue in patients with ST-elevation MI (STEMI), where patients with present or absent cardiac rupture were compared, found that $CD209^+$ DCs and $CD11^+$ DC infiltration was higher in patients with cardiac rupture, with a significant positive correlation between $CD209^+$ DCs $CD11c^+$ DCs and the extent of fibrosis further detected [58]. A more systematically designed study, where STEMI, non-STEMI (NSTEMI), and CAD ($n = 123$) patients were assessed for tissue-residing and circulating DCs [59], revealed circulating mDCs, pDCs, and total DCs decreased after acute MI, especially in STEMI patients, with higher DC numbers found in the infarcted myocardium. These results suggest that the lower numbers of circulating DCs after MI may be mediated by DC migration into the myocardium, which is indeed supported by several studies showing a reduction of circulating immature DCs [60], mDCs [61–63], or both, mDCs and pDCs [64] after MI. Further evidence shows that reduced circulating DC numbers return to baseline levels after seven days and do not change for a time period of three months later [64]. Moreover, the mDC/pDC ratio seems to be an additional important predictor to distinguish between coronary syndromes, as data has shown that a mDC/pDC ratio ≥ 4 allows patients suffering from acute coronary syndrome to

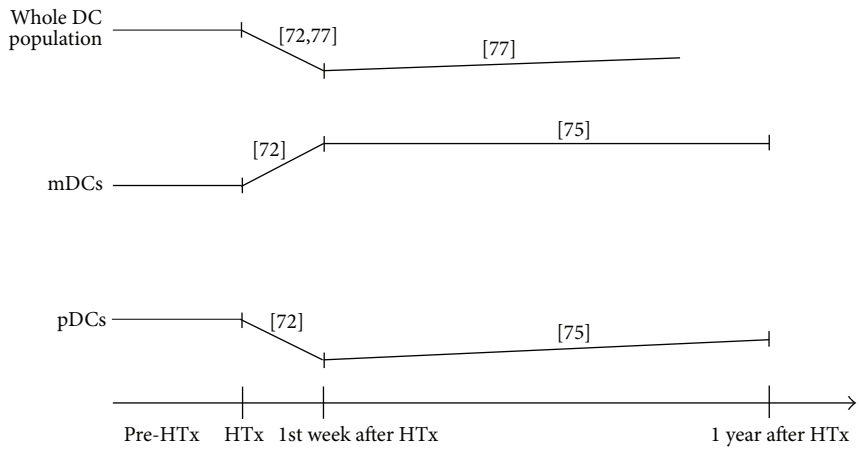


Figure 1: Changes of dendritic cells and their subsets following heart transplantation. Increases and decreases of the whole DC population and the subsets mDCs and pDCs following heart transplantation were visualized. The appropriate references were added in squared brackets. DCs: dendritic cells; HTx: heart transplantation; mDCs: myeloid dendritic cells; pDCs: plasmacytoid dendritic cells.

be separated compared to those with stable angina pectoris or healthy controls [62].

In contrast, other studies have suggested that autoimmunological processes occur after MI in consequence to defective peripheral tolerance, as autoantibodies against myosin heavy chain, troponins, and β1-adrenoreceptors have been found in patients with dilative cardiomyopathy (DCM) or HF [65–69]. Ischemia induces changes not only to DCs but also in DCM, where data shows that chronic DC-driven myocardial inflammation results in ventricular functional impairment with hemodynamic characteristics resembling DCM [70]. Pistulli and coworkers investigated 72 endomyocardial biopsies from patients with diagnosed DCM and found a reduction in both myocardial DCs of all subtypes (mDCs, pDCs, mature DCs, and immature DCs) and maturation markers (fascin, CD11c, CD209, CD83, and CD304), as well as an inverse correlation of DCs with tissue fibrosis. Furthermore, a reduction of mDCs in DCM hearts in concert with positive testing for cardiotropic viruses has been reported, which raises the hypothesis about a connection between mDCs and myocardial virus clearance [71].

The situation of DCs looks different in whole blood of patients with chronic HF (CHF) [72], where elevated mDC and mature DCs levels have been reported while pDCs were unchanged. It has been hypothesized that the shift of the mDC/pDC balance towards mature mDCs may be associated with T_H1 biased immune responses in later stages of CHF [72]. A few years after this study was published, Athanassopoulos et al. reported that patients with end-stage CHF of NYHA category III and category IV had comparable levels of circulating DC subsets to NYHA II patients and healthy volunteers [73]. In contrast, Sugi et al. showed that patients with NYHA III and NYHA IV had lower counts of circulating mDCs and pDCs [74]. After treatment of decompensated HF with optimized oral and intravenous heart insufficiency medication, the reduction of circulating mDCs and pDCs was restored and increased during the following weeks. Overall, these findings suggest that the role of DCs in the pathophysiology of HF is controversial, with further studies required to clarify the associations between DCs and disease development.

6. DCs and Heart Transplantation

The central role of DCs in mediating inflammation and immune tolerance has been demonstrated for MI, myocarditis, DCM, and HF. Aside from these diseases, DCs hold a key role in the immunological processes that are connected to allograft rejection. Inflammatory processes and allograft rejection belong to the major complications after heart transplantation (HTx), whereas immunological tolerance is closely related to a positive outcome following HTx. Clinical studies have focused on monitoring DCs after HTx, with the aim of investigating whether DCs are a valuable marker of immune function status after transplantation [75]. Athanassopoulos et al. examined total peripheral blood mDC and pDC subsets expressing CD83 and CCR7 in 16 patients before HTx and one week after HTx compared to 14 healthy controls [72]. A further study of this group investigated DCs and their subsets up to 38 weeks after HTx in 20 HTx patients [76]. Dieterlen et al. investigated DCs and mDC and pDC subsets in the first twelve months after HTx in 46 HTx patients [75]. In summary, these studies revealed that patients had higher percentage of DCs before transplantation (Figure 1). Within the first week after HTx, a marked decrease in both the percentage of DCs and that of pDCs was observed but an increase in the percentage of mDCs was observed [72, 76], with the number of the whole DC populations increasing continuously during the following months [76]. A more detailed analysis of DC subsets showed that pDCs increased during the first year after HTx, while mDCs remained constant within that time [75]. In summary, these studies demonstrated that DC incidence and subset distribution differed substantially between recipients before and after HTx as well as in healthy subjects. Thus, it has been stated that DC homeostasis is altered after transplantation (see Figure 1) [77].

Surgery and stress cause a transient increase of peripheral blood DCs [78, 79], and therefore the decrease of DCs and

their subsets in the early posttransplantation period has to be ascribed to the immunosuppressive treatment by antithymocyte globulin, corticosteroids, calcineurin inhibitors, or mycophenolate mofetil [72, 76]. The different effects and mechanisms of immunosuppressive drugs on DCs are shown in Table 1.

Immunosuppressive drugs keep DC subsets in an immature state, where there is a potent effect not only on maturation but also on the migration characteristics of mDCs and pDCs [90–92]. These findings are in accordance with a report on circulating DCs, which found lack in the expression of the maturation markers CD83 and CCR7 early after HTx [72]. Furthermore, it has been reported that mycophenolate mofetil influenced phenotype and function during the maturation process, and cyclosporine A and tacrolimus inhibited DC migration [93]. A direct comparison of cyclosporine A- (n = 14) and tacrolimus-treated (n = 14) HTx recipients showed that the percentage of mDC values was higher and percentage of pDC values was lower in the cyclosporine A-treated group than in the tacrolimus-treated group. Additionally, monitoring the same study cohort for DC subsets over a period of six months showed that mDC values only differed at study onset and aligned up to month 6. In contrast, pDC values and the pDC/mDC ratio differed significantly at all study time points (day 0, month 3, and month 6) [94]. Barten et al. monitored DCs in 16 HTx patients with regard to the immunosuppressive regimen after conversion of calcineurin inhibitors or sirolimus to everolimus [95]. Regardless of the immunosuppressive regimen, HTx patients had higher percentage of mDCs compared to healthy controls, whereas pDCs were only significantly lower in patients with conversion from calcineurin inhibitors to everolimus. Sirolimus maintenance therapy caused a similar percentage of pDCs compared to controls, with a shift to pDCs in the pDCs/mDCs ratio compared to recipients with calcineurin inhibitor therapy. Furthermore, an additional elevated shift in the pDC/mDC ratio towards pDCs after conversion from calcineurin inhibitors or sirolimus to everolimus has been observed, which was comparably higher than controls [95].

As mentioned above, DC monitoring is performed to investigate DCs as a marker of immune function status. Thus, studies often correlate DC numbers and acute cellular rejection (ACR). Two different study cohorts have been investigated regarding the influence of rejection episodes on DCs. Firstly, John et al. analysed twenty-eight HTx patients and found that the percentage of pDCs was lower in HTx recipients with rejection compared to HTx recipients without ACR. In contrast, no differences between rejector and nonrejectors have been detected for mDCs [94]. Secondly, a study cohort including twenty-one HTx patients correlated DC subsets with different rejection grades [76, 77]. A negative association of mDCs but not of pDCs with the rejection grade determined from endomyocardial biopsies has been found. The number of peripheral blood DCs and the mDC/pDC ratio decreased markedly during ACR episodes, and a lower mDC number has been documented even three months after ACR [77]. Furthermore, Athanassopoulos et al. showed that aberrant DC reconstitution is related to adverse clinical outcome after HTx [76].

The central role of DCs in immunological processes led to the development of cellular vaccination strategies aiming to induce transplant tolerance [96]. DCs that are involved in processes leading to tolerance were named "tolerogenic DCs" (tolDCs). tolDCs have immunosuppressive characteristics and exert their function via passive (lack of costimulatory signals) and active (presence of inhibitory signals) tolerance [97]. According to their organism of origin, tolDCs can be classified into "donor-derived tolDCs" and "recipient-derived tolDCs." Furthermore, it is possible to generate tolDCs *in vitro*, for example, from monocytes [98], or to induce tolDCs *in vivo*.

One possibility for the *in vivo* induction of tolDCs is extracorporeal photopheresis (ECP) [99]. ECP is an apheresis technique that collects a portion of patients' venous whole blood in a medical device located outside the patients' body (extracorporeal). After separating the blood into its components by centrifugation, the fraction containing white blood cells is treated with the photosensitizing drug methoxsalen and UV-A light and then returned into the patients' circulation. The investigation of DC subsets in HTx patients (n = 25) during and after ECP treatment showed that almost 80% of the treated HTx patients had increased pDCs and regulatory T cells (T_{regs}) [100]. The authors proposed classification criteria based on the individual courses of pDCs and T_{regs} to discriminate between patient specific responses to ECP therapy.

tolDCs induce tolerogenic immune reactions and immunomodulation faster and more frequently than immature DCs [97]. The mechanisms that are involved in immunomodulation are the IL-10- and TGF-β-driven differentiation of T_{regs}, the cytokine production that promotes tolDC biology, and the cytokine expression of inhibitory molecules that regulate T cell responses [97]. Animal models demonstrated that cardiac allograft survival is prolonged by infusion of tolerogenic pDCs in combination with anti-CD40L therapy [101, 102]. At present, no clinical application of tolDCs is approved or under investigation for HTx. The ONE Study, which is an ongoing multicenter, prospective, and randomized clinical trial, is the first study that evaluates immunomodulatory cellular therapy of ECP on tolDCs in kidney transplantation [103].

Irrespectively of the *in vitro* generation of tolDCs, different types of tolDCs have been found in humans *in vivo* [97]. DC-10 cells, a type of mDC expressing IL-10, have been identified by Gregori et al. [104]. This type of tolDC expressed inhibitory molecules (ILT-2, ILT-3, and ILT-4) and the costimulatory surface molecules CD40 and CD86 and trigger tolerogenic effects [104]. Natural tolerogenic pDCs differed in their tolerance-inducing properties compared to tolerogenic mDCs. These differences are caused by the biology of pDCs, which includes a less effective antigen presentation, different maturation characteristics, and expression of costimulatory molecules [105].

7. Conclusions

We have reviewed the role and alterations of DCs in CVD and also the current state-of-the-art research. While there remain numerous gaps and contrary findings related to the effects of

Table 1: Effects of immunosuppressive drugs on dendritic cells.

Immunosuppressive drug	Effect on DCs	Reference
Anti-thymocyte globulin	(i) Binding of immature and mature DC subsets with the following induction of complement-mediated DC lysis	Monti et al., 2003 [80]
Cyclosporine A	(i) Interference with DC recirculation through cyclooxygenase-2 inhibition or prostaglandin E2 uncoupling with CCR7	Luft et al., 2002 [81]; Scandella et al., 2004 [82]
	(ii) Inhibition of DC migration by competitive inhibition of the lipid transporters MDR1 and MRP1	Randolph, 2001 [83]
	(iii) Inhibition of NFκB production in DCs	Szabo et al., 2001 [84]
Sirolimus	(i) Inhibition of mDCs' IL-12 signaling	Chiang et al., 2004 [85]
Tacrolimus	(i) Suppression of DC allocostimulatory capacity by decreasing TNF-α and IL-12 secretion	Lagaraine and Lebranchu, 2003 [86]
	(ii) Inhibition of NFκB production in DCs	Szabo et al., 2001 [84]
Mycophenolate mofetil	(i) Suppression of DC allocostimulatory capacity by decreasing TNF-α and IL-12 secretion	Lagaraine and Lebranchu, 2003 [86]
Prednisone/Dexamethasone	(i) Reduction of circulating pDC numbers	Moser et al., 1995 [87]
	(ii) Induction of pDC apoptosis	Boor et al., 2006 [88]
	(iii) Inhibition of DC function *in vivo*	Shodell and Siegal, 2001 [89]
	(iv) Inhibition of DC migration by competitive inhibition of the lipid transporters MDR1 and MRP1	Randolph, 2001 [83]

CCR7: chemokine receptor 7; DC/DCs: dendritic cell/dendritic cells; IL-12: interleukin-12; MDR1: multidrug resistance 1; MRP1: multidrug resistance protein; NFκB: nuclear factor "kappa-light-chain-enhancer" of activated B-cells; pDC: plasmacytoid dendritic cell; TNF-α: tumor necrosis factor-α.

Table 2: Clinical studies on the role of dendritic cells in cardiovascular diseases.

Study	Type of cardiovascular disease	Study population	Major findings
	Atherosclerosis		
Cherian et al., 2000 [107]	Atherosclerosis	Patients with aortocoronary bypass ($n = 12$)	(i) DCs present in stenotic aortocoronary saphenous vein bypass grafts
Cherian et al., 2001 [35]	Atherosclerosis	Patients with aortocoronary bypass ($n = 14$) and healthy controls ($n = 10$)	(i) $CD1a^+/S100^+$ DCs present in stenotic saphenous vein bypass grafts but not in normal saphenous veins
Ozmen et al., 2001 [38]	Atherosclerosis	Patients with stenotic aortocoronary saphenous vein grafts ($n = 3$) and control carotid arteries ($n = 8$)	(i) $CD40^+$ cells detected in stenotic grafts and carotid plaques (ii) $CD40^+/S100^+$ cells clustered within the intima, the media, and the adventitia
Yilmaz et al., 2006 [29]	Atherosclerosis	Patients with carotid endarterectomy ($n = 44$)	(i) Lower DC numbers in initial lesions than in advanced plaques (ii) DC number higher in stable than in vulnerable plaques (iii) 70% of DCs in advanced plaques with mature phenotype indicate functional activity of DCs
Van Vré et al., 2006 [30]	Atherosclerosis	CAD patients ($n = 18$) and controls ($n = 18$)	(i) Lower numbers and percentage of pDCs and mDCs in patients with CAD than in controls
Niessner et al., 2006 and 2007 [24, 25]	Atherosclerosis	Patients with carotid endarterectomy ($n = 30$)	(i) 53% of carotid samples with $CD123^+$ pDCs and with $CD11c^+$ DC-$Sign^+$ $fascin^+$ mDCs (ii) DCs localized in the shoulder region and at the base of the plaque (iii) pDCs are localized in the shoulder region and produce IFN-α (iv) IFN-α transcript concentrations correlated with plaque instability (v) mDC : pDC ratio of 2.7 in the plaques
Erbel et al., 2007 [28]	Atherosclerosis	Patients with carotid artery plaques ($n = 57$)	(i) Plaques from patients with ischemic complications with elevated levels of CD83, CCL19, and CCL21 (ii) Presence of $CD83^+$ DCs in the shoulder region of unstable plaques
Yilmaz et al., 2009 [31]	Atherosclerosis	CAD patients ($n = 290$)	(i) Reduction of pDCs, mDCs, and DCs in advanced CAD patients (ii) Reduction of pDCs, mDCs, and DCs in patients with required percutaneous coronary intervention or coronary artery bypass grafting
Van Vré et al., 2010 [32]	Atherosclerosis	CAD patients ($n = 46$) and controls ($n = 15$)	(i) Decrease of total blood DCs, mDCs, and pDCs in CAD patients compared to controls (ii) Inverse association of IL-6 and hs-CRP with mDCs
Van Vré et al., 2011 [27]	Atherosclerosis	Patients with carotid endarterectomy ($n = 22$) or autopsy ($n = 87$)	(i) Accumulation of BDCA-1 and BDCA-2 near microvessels (ii) $S100^+$ and $fascin^+$ DCs increased from intimal thickening via pathological thickening, fibrous cap atheroma to complicated plaques
Van Brussel et al., 2011 [33]	Atherosclerosis	CAD patients ($n = 15$) and controls ($n = 12$)	(i) Circulating mDCs and pDCs declined in CAD patients (ii) Frequencies of $CD86^+$ and $CCR7^+$ mDCs, but not pDCs, declined in CAD patients (iii) Plasma Flt3L positively correlated with blood DC counts
Abbas et al., 2015 [34]	Atherosclerosis	Patients with carotid atherosclerosis ($n = 177$) and healthy controls ($n = 24$)	(i) pDCs with increased mRNA levels of IL-23 and IL-23R in atherosclerosis
Rohm et al., 2015 [26]	Atherosclerosis	Patients with carotid endarterectomy ($n = 29$)	(i) Higher numbers of $fascin^+$, $S100^+$, or $CD83^+$ mDCs are unstable compared with stable plaques (ii) No differences between stable and unstable plaques for pDCs
	Hypertension		
Kirabo et al., 2014 [47]	Hypertension	Hypertensive patients ($n = 142$) and normotensive controls ($n = 24$)	(i) Elevated levels of isoketal-modified proteins in circulating monocytes and DCs in patients with hypertension (ii) Hypertension activates DCs, in large part by promoting the formation of isoketals

Table 2: Continued.

Study	Type of cardiovascular disease	Study population	Major findings
	Heart failure associated diseases		
Yokoyama et al., 2000 [5]	Myocarditis	Acute myocarditis patients ($n = 22$) and patients that died from noncardiac disease ($n = 20$)	(i) Cardiac DCs increase in the acute phase of myocarditis (ii) Cardiac DCs with long, slender dendritic processes and positive for HLA-DR, but negative for CD68
Athanassopoulos et al., 2004 [72]	HF, transplantation	HF/HTx patients ($n = 16$) and healthy controls ($n = 14$)	(i) Increase of blood DCs and mDCs in CHF patients (ii) Increase of mature DC subsets compared to controls
Yilmaz et al., 2006 [29]	MI	Angina pectoris ($n = 39$) and MI ($n = 17$) patients	(i) Reduced circulating mDCs in patients with angina pectoris and acute myocardial infarction compared to controls (ii) mDCs inversely correlated with C-reactive protein or IL-6 (iii) More mDC precursors in vulnerable carotid plaques than in stable ones
Athanassopoulos et al., 2009 [73]	HF	NYHA II patients ($n = 12$), NYHA III/IV patients ($n = 28$), and healthy controls ($n = 18$)	(i) NYHA III/IV patients with comparable percentage of circulating DC subsets (ii) Within NYHA III/IV patients: total DC levels in patients with nonischemic DCM higher than in patients with CAD, HF, and HCM (iii) Mature mDCs, but not pDCs, in DCM patients compared to patients with CAD, HCM, or other cardiac pathophysiologies
Sugi et al., 2011 [74]	HF	Patients with decompensated HF ($n = 27$)	(i) Circulating DC subsets lower in decompensated HF patients compared to controls (ii) HF treatment restored reduction and activation of circulating mDCs and pDCs (iii) Numbers of circulating DCs correlated with decreases of BNP and troponin-T (iv) Poor recovery of circulating DC numbers predictive of recurrence of decompensated HF
Kofler et al., 2011 [60]	MI	STEMI patients ($n = 35$), NSTEMI patients ($n = 30$), stable CAD patients ($n = 15$), and controls ($n = 15$)	(i) Downregulation of immature ($CD1a^+$) DCs in STEMI, NSTEMI, and CAD patients (ii) Upregulation of mature ($CD86^+$) DCs in CAD patients
Fukui et al., 2012 [64]	MI	AMI patients ($n = 26$), SAP patients ($n = 19$), and controls ($n = 19$)	(i) Circulating mDCs and pDCs lower in AMI group than in SAP or control group (ii) Numbers of circulating mDCs and pDCs returned to control levels 7 days after AMI and were stable until the next 3 months (iii) % $CD40^+$ and $CD83^+$ mDCs higher in AMI patients than in SAP group or controls (iv) % $CD40^+$ and $CD83^+$ pDCs were similar between the three groups
Carvalheiro et al., 2012 [63]	MI	AMI patients ($n = 12$) and healthy controls ($n = 12$)	(i) Lower frequency of circulating mDCs
Kretzschmar et al., 2012 [59]	MI	STEMI patients ($n = 34$), NSTEMI patients ($n = 44$), and controls ($n = 45$)	(i) Decrease of circulating mDCPs, pDCPs, and tDCPs in AMI patients with pronounced reduction in STEMI patients (ii) Higher DC number in infarcted myocardium than in control
Wen et al., 2013 [61]	MI	AMI patients ($n = 50$), SAP patients ($n = 30$), UAP patients ($n = 56$), and controls ($n = 29$)	(i) % circulating mDC precursors reduced in AMI and UAP patients compared to SAP patients and controls (ii) % circulating pDC precursors not different between the groups (iii) % circulating mDC precursors negatively correlated with severity and extent of coronary artery lesions
Pistulli et al., 2013 [71]	DCM	DCM patients ($n = 72$)	(i) Myocardial DCs of all subtypes and maturation stages decreased in DCM compared to controls (ii) T_{regs}, apoptosis, and CCR7 overexpressed in DCM (iii) mDCs reduced in virus-positive endomyocardial biopsies (iv) mDC number correlated with positive change in EF at follow-up
Nagai et al., 2014 [58]	MI	STEMI patients with present ($n = 13$) or absent ($n = 11$) cardiac rupture	(i) $CD209^+$ DC and $CD11c^+$ DC infiltration increased in the rupture group (ii) Positive correlation between the number of infiltrating $CD209^+$ DCs and $CD11c^+$ DCs and the extent of reparative fibrosis

Table 2: Continued.

Study	Type of cardiovascular disease	Study population	Major findings
	Transplantation		
Athanassopoulos et al., 2004 [72]	Transplantation, HF	HTx patients ($n = 16$) and healthy controls ($n = 14$)	(i) Decrease of total DCs, mDCs, and pDCs one week after HTx (ii) % of circulating mDCs higher after HTx compared to CHF patients and controls (iii) Maturation status of DC subsets comparable to controls (but not the $CCR7^+$ pDCs)
Athanassopoulos et al., 2005 [76]	Transplantation	HTx patients ($n = 21$)	(i) Reduced DC numbers up to week 38 after HTx (ii) Negative association of mDCs with rejection grade (iii) mDCs and their mature states decreased during AR episodes and are lower in rejectors than in nonrejectors
Athanassopoulos et al., 2005 [77]	Transplantation	HTx patients ($n = 20$ venous blood analyses; $n = 14$ EMB analyses)	(i) Total DC numbers decreased at the first week after HTx and remained lower than the pre-HTx condition until week 38 (ii) Negative association between mDCs, but not pDCs, and the diagnosed ISHLT rejection grade for the follow-up period
Barten et al., 2006 [95]	Transplantation	HTx patients ($n = 16$) and healthy controls ($n = 20$)	(i) Higher % of mDCs in HTx patients compared to controls (ii) % of pDCs were different in patients with conversion from calcineurin inhibitors to everolimus compared to healthy controls (iii) Mature mDCs did not differ between HTx patients and controls
John et al., 2014 [94]	Transplantation	HTx patients ($n = 28$)	(i) mDCs higher and pDCs lower in cyclosporine A-treated patients than in tacrolimus-treated patients (ii) pDC/mDC ratio higher at day 0, month 3, and month 6 in tacrolimus-treated patients than in cyclosporine A-treated patients
Dieterlen et al., 2015 [75]	Transplantation	HTx patients ($n = 46$)	(i) Increase of pDCs, but not for mDCs, in the first year after HTx (ii) No significant changes of the pDC/mDC ratio in the first year after HTx

AMI: acute myocardial infarction; AR: acute rejection; BDCA-1/BDCA-2/BDCA-3/BDCA-4: blood dendritic cell antigen-1/antigen-2/antigen-3/antigen-4; BNP: B-type natriuretic peptide; CAD: coronary artery disease; CD1a/CD11c/CD40/CD68/CD83/CD123/CD209: Cluster of Differentiation 1a/11c/40/68/83/123/209; CHF: chronic heart failure; CCL19/CCL21: chemokine ligands 19/21; DCM: dilative cardiomyopathy; DC/DCs: dendritic cell/dendritic cells; EF: ejection fraction; EMB: endomyocardial biopsy; Flt3L: FMS-like tyrosine kinase 3 ligand; HCM: hypertrophic cardiomyopathy; HF: heart failure; HLA-DR: human leukocyte antigen DR; hs-CRP: high sensitivity C-reactive protein; HTx: heart transplantation; IFN-α: interferon-α; IL-6/IL-23/IL-23R: interleukin 6/interleukin 23/interleukin 23-receptor; ISHLT: International Society of Heart and Lung Transplantation; mDCs: myeloid dendritic cells; mDCPs: myeloid dendritic cell precursors; MI: myocardial infarction; NSTEMI: non-ST-elevation myocardial infarction; NYHA II/III/IV: New York Heart Association grade II/III/IV; pDCs: plasmacytoid dendritic cells; pDCPs: plasmacytoid dendritic cell precursors; SAP: stable angina pectoris; STEMI: ST-elevation myocardial infarction; S100: calcium-binding protein with low molecular weight, marker for DCs; tDCPs: total dendritic cell precursors; T_{regs}: regulatory T cells; UAP: unstable angina pectoris.

DCs in different CVDs, many observations of human studies are based on circulating measurements despite distinct DCs residing in the tissue that are not detected in analyses of peripheral blood samples. Therefore, analyses of circulating DCs fail to provide information about the processes that are initiated after DC activation or tissue-specific DCs. Such limitations can be closed with further intensive preclinical and clinical research, which should include studies measuring circulating and tissue-residing DCs simultaneously. An interesting aspect, which is yet to be studied in prospective clinical trials, is the role of circulating DCs as immunological markers for CVDs.

While circulating DCs can be analysed by flow cytometry, tissue-residing DCs are measurable by immunohistochemistry or slide-based cytometry [106]. However, it has to be noted that the investigation of tissue-residing DCs requires biopsy material, which may pose additional risks for patients dependent on CVDs. While it is clinical routine to perform endomyocardial biopsies to detect graft rejection after HTx, this is not the case for patients with MI and atherosclerosis.

At present, only few human studies with low patient numbers compared DCs in the tissue with peripheral blood DCs (Table 2). In particular, the role of DCs in hypertension and in diseases leading to HF is still widely unexplored. Furthermore, many studies present conflicting results, which may be related to the different markers used for DC classification, as some study groups favor the classification via the expression of CD11 and others define DC subsets by the markers BDCA1–BDCA4, DC-1a, or S100. Thus, a consensus is urgently required on the functional and phenotypical DC classification in order to allow results to become more comparable.

Furthermore, the number of circulating DCs is extremely small with less than 1% of the leukocytes being DC subsets. Therefore, by using detection methods that are designed to identify very rare cell populations, such as flow cytometry, for example, this problem could be dramatically reduced. However, the establishment of high-throughput methods for clinical diagnostics of DCs is hindered for rare cell populations.

In conclusion, while DCs represent a cell type capable of modulating immunological processes in CVDs, only clinical studies investigating both the circulating and tissue-residing DCs will help further clarify the underlying mechanisms of how these cells exert their immunological effects in humans.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] S. Mendis, P. Puska, and B. Norrving, *Global Atlas on Cardiovascular Disease Prevention and Control*, WHO, Geneva, Switzerland, 2011.

[2] D. A. Chistiakov, I. A. Sobenin, A. N. Orekhov, and Y. V. Bobryshev, "Myeloid dendritic cells: development, functions, and role in atherosclerotic inflammation," *Immunobiology*, vol. 220, pp. 833–844, 2015.

[3] A. Dzionek, A. Fuchs, P. Schmidt et al., "BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood," *The Journal of Immunology*, vol. 165, no. 11, pp. 6037–6046, 2000.

[4] J. Narbutt, A. Lesiak, M. Zak-Prelich et al., "The distribution of peripheral blood dendritic cells assayed by a new panel of anti-BDCA monoclonal antibodies in healthy representatives of the Polish population," *Cellular and Molecular Biology Letters*, vol. 9, no. 3, pp. 497–509, 2004.

[5] H. Yokoyama, S. Kuwao, K. Kohno, K. Suzuki, T. Kameya, and T. Izumi, "Cardiac dendritic cells and acute myocarditis in the human heart," *Japanese Circulation Journal*, vol. 64, no. 1, pp. 57–64, 2000.

[6] D. N. J. Hart and J. W. Fabre, "Demonstration and characterization of Ia-positive dendritic cells in the interstitial connective tissue of rat heart and other tissues, but not brain," *The Journal of Experimental Medicine*, vol. 154, no. 2, pp. 347–361, 1981.

[7] J. Zhang, Z.-X. Yu, S. Fujita, M. L. Yamaguchi, and V. J. Ferrans, "Interstitial dendritic cells of the rat heart. Quantitative and ultrastructural changes in experimental myocardial infarction," *Circulation*, vol. 87, no. 3, pp. 909–920, 1993.

[8] J. Banchereau, F. Briere, C. Caux et al., "Immunobiology of dendritic cells," *Annual Review of Immunology*, vol. 18, pp. 767–811, 2000.

[9] F. Sallusto, B. Palermo, D. Lenig et al., "Distinct patterns and kinetics of chemokine production regulate dendritic cell function," *European Journal of Immunology*, vol. 29, no. 5, pp. 1617–1625, 1999.

[10] C. Delgado-Martín, C. Escribano, J. L. Pablos, L. Riol-Blanco, and J. L. Rodríguez-Fernández, "Chemokine CXCL12 uses CXCR4 and a signaling core formed by bifunctional akt, Extracellular Signal-regulated Kinase (ERK)1/2, and Mammalian Target of Rapamycin Complex 1 (mTORC1) proteins to control chemotaxis and survival simultaneously in mature dendritic cells," *The Journal of Biological Chemistry*, vol. 286, no. 43, pp. 37222–37236, 2011.

[11] G. J. Randolph, J. Ochando, and S. Partida-Sánchez, "Migration of dendritic cell subsets and their precursors," *Annual Review of Immunology*, vol. 26, pp. 293–316, 2008.

[12] J. M. Curtsinger and M. F. Mescher, "Inflammatory cytokines as a third signal for T cell activation," *Current Opinion in Immunology*, vol. 22, no. 3, pp. 333–340, 2010.

[13] J. Frostegård, "Immunity, atherosclerosis and cardiovascular disease," *BMC Medicine*, vol. 11, article 117, 2013.

[14] M. Weis, C. L. Schlichting, E. G. Engleman, and J. P. Cooke, "Endothelial determinants of dendritic cell adhesion and migration: new implications for vascular diseases," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 22, no. 11, pp. 1817–1823, 2002.

[15] G. K. Hansson, "Inflammation, atherosclerosis, and coronary artery disease," *The New England Journal of Medicine*, vol. 352, no. 16, pp. 1626–1695, 2005.

[16] J. Frostegård, A.-K. Ulfgren, P. Nyberg et al., "Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines," *Atherosclerosis*, vol. 145, no. 1, pp. 33–43, 1999.

[17] L. S. Deluca and J. L. Gommerman, "Fine-tuning of dendritic cell biology by the TNF superfamily," *Nature Reviews Immunology*, vol. 12, no. 5, pp. 339–351, 2012.

[18] C. J. J. Alderman, P. R. Bunyard, B. M. Chain, J. C. Foreman, D. S. Leake, and D. R. Katz, "Effects of oxidised low density

lipoprotein on dendritic cells: a possible immunoregulatory component of the atherogenic micro-environment?" *Cardiovascular Research*, vol. 55, no. 4, pp. 806–819, 2002.

[19] J. Ge, Q. Jia, C. Liang et al., "Advanced glycosylation end products might promote atherosclerosis through inducing the immune maturation of dendritic cells," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 10, pp. 2157–2163, 2005.

[20] A. Aicher, C. Heeschen, M. Mohaupt, J. P. Cooke, A. M. Zeiher, and S. Dimmeler, "Nicotine strongly activates dendritic cell-mediated adaptive immunity: potential role for progression of atherosclerotic lesions," *Circulation*, vol. 107, no. 4, pp. 604–611, 2003.

[21] H. Lu, D. Huang, K. Yao et al., "Insulin enhances dendritic cell maturation and scavenger receptor-mediated uptake of oxidised low-density lipoprotein," *Journal of Diabetes and Its Complications*, vol. 29, no. 4, pp. 465–471, 2015.

[22] W. Nie, H. Yan, S. Li, W. Zhu, F. Fan, and J. Zhu, "Angiotensin II promotes atherogenesis through upregulating the expression of connexin 43 in dendritic cells," *Cellular and Molecular Biology (Noisy-le-Grand)*, vol. 61, no. 3, pp. 96–101, 2015.

[23] A. Yilmaz, M. Lochno, F. Traeg et al., "Emergence of dendritic cells in rupture-prone regions of vulnerable carotid plaques," *Atherosclerosis*, vol. 176, no. 1, pp. 101–110, 2004.

[24] A. Niessner, K. Sato, E. L. Chaikof, I. Colmegna, J. J. Goronzy, and C. M. Weyand, "Pathogen-sensing plasmacytoid dendritic cells stimulate cytotoxic T-cell function in the atherosclerotic plaque through interferon-α," *Circulation*, vol. 114, no. 23, pp. 2482–2489, 2006.

[25] A. Niessner, M. S. Shin, O. Pryshchep, J. J. Goronzy, E. L. Chaikof, and C. M. Weyand, "Synergistic proinflammatory effects of the antiviral cytokine interferon-α and toll-like receptor 4 ligands in the atherosclerotic plaque," *Circulation*, vol. 116, no. 18, pp. 2043–2052, 2007.

[26] I. Rohm, Y. Atiskova, S. Drobnik et al., "Decreased regulatory T cells in vulnerable atherosclerotic lesions: imbalance between pro- and anti-inflammatory cells in atherosclerosis," *Mediators of Inflammation*, vol. 2015, Article ID 364710, 13 pages, 2015.

[27] E. A. Van Vré, J. M. Bosmans, I. Van Brussel et al., "Immunohistochemical characterisation of dendritic cells in human atherosclerotic lesions: possible pitfalls," *Pathology*, vol. 43, no. 3, pp. 239–247, 2011.

[28] C. Erbel, K. Sato, F. B. Meyer et al., "Functional profile of activated dendritic cells in unstable atherosclerotic plaque," *Basic Research in Cardiology*, vol. 102, no. 2, pp. 123–132, 2007.

[29] A. Yilmaz, J. Weber, I. Cicha et al., "Decrease in circulating myeloid dendritic cell precursors in coronary artery disease," *Journal of the American College of Cardiology*, vol. 48, no. 1, pp. 70–80, 2006.

[30] E. A. Van Vré, V. Y. Hoymans, H. Bult et al., "Decreased number of circulating plasmacytoid dendritic cells in patients with atherosclerotic coronary artery disease," *Coronary Artery Disease*, vol. 17, no. 3, pp. 243–248, 2006.

[31] A. Yilmaz, T. Schaller, I. Cicha et al., "Predictive value of the decrease in circulating dendritic cell precursors in stable coronary artery disease," *Clinical Science*, vol. 116, no. 4, pp. 353–363, 2009.

[32] E. A. Van Vré, I. Van Brussel, K. O. de Beeck et al., "Changes in blood dendritic cell counts in relation to type of coronary artery disease and brachial endothelial cell function," *Coronary Artery Disease*, vol. 21, no. 2, pp. 87–96, 2010.

[33] I. Van Brussel, E. A. Van Vré, G. R. Y. De Meyer, C. J. Vrints, J. M. Bosmans, and H. Bult, "Decreased numbers of peripheral blood dendritic cells in patients with coronary artery disease are associated with diminished plasma Flt3 ligand levels and impaired plasmacytoid dendritic cell function," *Clinical Science*, vol. 120, no. 9, pp. 415–426, 2011.

[34] A. Abbas, I. Gregersen, S. Holm et al., "Interleukin 23 levels are increased in carotid atherosclerosis: possible role for the interleukin 23/interleukin 17 axis," *Stroke*, vol. 46, pp. 793–799, 2015.

[35] S. M. Cherian, Y. V. Bobryshev, H. Liang et al., "Immunohistochemical and ultrastructural evidence that dendritic cells infiltrate stenotic aortocoronary saphenous vein bypass grafts," *Cardiovascular Surgery*, vol. 9, no. 2, pp. 194–200, 2001.

[36] B. H. Bulkley and G. M. Hutchins, "Accelerated 'atherosclerosis'. A morphologic study of 97 saphenous vein coronary artery bypass grafts," *Circulation*, vol. 55, no. 1, pp. 163–169, 1977.

[37] L. Campeau, M. Enjalbert, J. Lespérance, C. Vaislic, C. M. Grondin, and M. G. Bourassa, "Atherosclerosis and late closure of aortocoronary saphenous vein grafts: sequential angiographic studies at 2 weeks, 1 year, 5 to 7 years, and 10 to 12 years after surgery," *Circulation*, vol. 68, no. 3, part 2, pp. II1–II7, 1983.

[38] J. Ozmen, Y. V. Bobryshev, and R. S. A. Lord, "CD40 costimulatory molecule expression by dendritic cells in primary atherosclerotic lesions in carotid arteries and in stenotic saphenous vein coronary artery grafts," *Cardiovascular Surgery*, vol. 9, no. 4, pp. 329–333, 2001.

[39] G. Wick, M. Romen, A. Amberger et al., "Atherosclerosis, autoimmunity, and vascular-associated lymphoid tissue," *The FASEB Journal*, vol. 11, no. 13, pp. 1199–1207, 1997.

[40] P. S. Yusuf, S. Hawken, S. Ôunpuu et al., "Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study," *The Lancet*, vol. 364, no. 9438, pp. 937–952, 2004.

[41] D. N. Muller, E. Shagdarsuren, J.-K. Park et al., "Immunosuppressive treatment protects against angiotensin II-induced renal damage," *The American Journal of Pathology*, vol. 161, no. 5, pp. 1679–1693, 2002.

[42] C. Nataraj, M. I. Oliverio, R. B. Mannon et al., "Angiotensin II regulates cellular immune responses through a calcineurin-dependent pathway," *The Journal of Clinical Investigation*, vol. 104, no. 12, pp. 1693–1701, 1999.

[43] M. S. Madhur, H. E. Lob, L. A. McCann et al., "Interleukin 17 promotes angiotensin II–induced hypertension and vascular dysfunction," *Hypertension*, vol. 55, no. 2, pp. 500–507, 2010.

[44] S. D. Crowley, Y.-S. Song, E. E. Lin, R. Griffiths, H.-S. Kim, and P. Ruiz, "Lymphocyte responses exacerbate angiotensin II-dependent hypertension," *The American Journal of Physiology—Regulatory Integrative and Comparative Physiology*, vol. 298, no. 4, pp. R1089–R1097, 2010.

[45] T. J. Guzik, N. E. Hoch, K. A. Brown et al., "Role of the T cell in the genesis of angiotensin II-induced hypertension and vascular dysfunction," *Journal of Experimental Medicine*, vol. 204, no. 10, pp. 2449–2460, 2007.

[46] H. Nguyen, V. L. Chiasson, P. Chatterjee, S. E. Kopriva, K. J. Young, and B. M. Mitchell, "Interleukin-17 causes Rho-kinase-mediated endothelial dysfunction and hypertension," *Cardiovascular Research*, vol. 97, no. 4, pp. 696–704, 2013.

[47] A. Kirabo, V. Fontana, A. P. C. de Faria et al., "DC isoketal-modified proteins activate T cells and promote hypertension," *The Journal of Clinical Investigation*, vol. 124, no. 10, pp. 4642–4656, 2014.

[48] S. Sagar, P. P. Liu, and L. T. Cooper Jr., "Myocarditis," *The Lancet*, vol. 379, no. 9817, pp. 738–747, 2012.

[49] G. K. Griffin and A. H. Lichtman, "Two sides to every proinflammatory coin: new insights into the role of dendritic cells in the regulation of T-cell driven autoimmune myocarditis," *Circulation*, vol. 127, no. 23, pp. 2257–2260, 2013.

[50] R. R. Marty and U. Eriksson, "Dendritic cells and autoimmune heart failure," *International Journal of Cardiology*, vol. 112, no. 1, pp. 34–39, 2006.

[51] N. R. Rose, "Infection, mimics, and autoimmune disease," *The Journal of Clinical Investigation*, vol. 107, no. 8, pp. 943–944, 2001.

[52] K. Bachmaier, N. Nikolaus, L. M. de la Maza, P. Sukumar, A. Hessel, and J. M. Penninger, "Chlamydia infections and heart disease linked through antigenic mimicry," *Science*, vol. 283, no. 5406, pp. 1335–1339, 1999.

[53] U. Hofmann and S. Frantz, "Role of lymphocytes in myocardial injury, healing, and remodeling after myocardial infarction," *Circulation Research*, vol. 116, no. 2, pp. 354–367, 2015.

[54] A. Anzai, T. Anzai, S. Nagai et al., "Regulatory role of dendritic cells in postinfarction healing and left ventricular remodeling," *Circulation*, vol. 125, no. 10, pp. 1234–1245, 2012.

[55] K. Naito, T. Anzai, Y. Sugano et al., "Differential effects of GM-CSF and G-CSF on infiltration of dendritic cells during early left ventricular remodeling after myocardial infarction," *Journal of Immunology*, vol. 181, no. 8, pp. 5691–5701, 2008.

[56] U. Hofmann, N. Beyersdorf, J. Weirather et al., "Activation of $CD4^+$ T lymphocytes improves wound healing and survival after experimental myocardial infarction in mice," *Circulation*, vol. 125, no. 13, pp. 1652–1663, 2012.

[57] H. Liu, W. Gao, J. Yuan et al., "Exosomes derived from dendritic cells improve cardiac function via activation of $CD4^+$ T lymphocytes after myocardial infarction," *Journal of Molecular and Cellular Cardiology*, vol. 91, pp. 123–133, 2016.

[58] T. Nagai, S. Honda, Y. Sugano et al., "Decreased myocardial dendritic cells is associated with impaired reparative fibrosis and development of cardiac rupture after myocardial infarction in humans," *Journal of the American Heart Association*, vol. 3, Article ID e000839, 2014.

[59] D. Kretzschmar, S. Betge, A. Windisch et al., "Recruitment of circulating dendritic cell precursors into the infarcted myocardium and pro-inflammatory response in acute myocardial infarction," *Clinical Science*, vol. 123, no. 6, pp. 387–398, 2012.

[60] S. Kofler, Z. Sisic, N. Shvets, P. Lohse, and M. Weis, "Expression of circulatory dendritic cells and regulatory T-cells in patients with different subsets of coronary artery disease," *Journal of Cardiovascular Pharmacology*, vol. 57, no. 5, pp. 542–549, 2011.

[61] J. Wen, Y. Wen, L. Zhiliang et al., "A decrease in the percentage of circulating mDC precursors in patients with coronary heart disease: a relation to the severity and extent of coronary artery lesions?" *Heart and Vessels*, vol. 28, no. 2, pp. 135–142, 2013.

[62] T. Fukunaga, H. Soejima, A. Irie et al., "High ratio of myeloid dendritic cells to plasmacytoid dendritic cells in blood of patients with acute coronary syndrome," *Circulation Journal*, vol. 73, no. 10, pp. 1914–1919, 2009.

[63] T. Carvalheiro, I. Velada, A. Valado et al., "Phenotypic and functional alterations on inflammatory peripheral blood cells after acute myocardial infarction," *Journal of Cardiovascular Translational Research*, vol. 5, no. 3, pp. 309–320, 2012.

[64] D. Fukui, H. Yasukawa, Y. Sugi et al., "Transient reduction and activation of circulating dendritic cells in patients with acute myocardial infarction," *International Journal of Cardiology*, vol. 160, no. 3, pp. 216–219, 2012.

[65] U. Eriksson, R. Ricci, L. Hunziker et al., "Dendritic cell-induced autoimmune heart failure requires cooperation between adaptive and innate immunity," *Nature Medicine*, vol. 9, no. 12, pp. 1484–1490, 2003.

[66] A. L. P. Caforio, M. Grazzini, J. M. Mann et al., "Identification of α- and β-cardiac myosin heavy chain isoforms as major autoantigens in dilated cardiomyopathy," *Circulation*, vol. 85, no. 5, pp. 1734–1742, 1992.

[67] Z. Kaya, C. Leib, and H. A. Katus, "Autoantibodies in heart failure and cardiac dysfunction," *Circulation Research*, vol. 110, no. 1, pp. 145–158, 2012.

[68] F. Leuschner, J. Li, S. Göser et al., "Absence of auto-antibodies against cardiac troponin I predicts improvement of left ventricular function after acute myocardial infarction," *European Heart Journal*, vol. 29, no. 16, pp. 1949–1955, 2008.

[69] V. Boivin-Jahns, A. Schlipp, S. Hartmann et al., "Antibodies to cardiac receptors," *Herz*, vol. 37, no. 8, pp. 843–848, 2012.

[70] M. Afanasyeva, D. Georgakopoulos, D. F. Belardi et al., "Quantitative analysis of myocardial inflammation by flow cytometry in murine autoimmune myocarditis: correlation with cardiac function," *The American Journal of Pathology*, vol. 164, no. 3, pp. 807–815, 2004.

[71] R. Pistulli, S. König, S. Drobnik et al., "Decrease in dendritic cells in endomyocardial biopsies of human dilated cardiomyopathy," *European Journal of Heart Failure*, vol. 15, no. 9, pp. 974–985, 2013.

[72] P. Athanassopoulos, L. M. B. Vaessen, A. P. W. M. Maat, A. H. M. M. Balk, W. Weimar, and A. J. J. C. Bogers, "Peripheral blood dendritic cells in human end-stage heart failure and the early post-transplant period: evidence for systemic Th1 immune responses," *European Journal of Cardio-Thoracic Surgery*, vol. 25, no. 4, pp. 619–626, 2004.

[73] P. Athanassopoulos, A. H. M. M. Balk, L. M. B. Vaessen et al., "Blood dendritic cell levels and phenotypic characteristics in relation to etiology of end-stage heart failure: implications for dilated cardiomyopathy," *International Journal of Cardiology*, vol. 131, no. 2, pp. 246–256, 2009.

[74] Y. Sugi, H. Yasukawa, H. Kai et al., "Reduction and activation of circulating dendritic cells in patients with decompensated heart failure," *International Journal of Cardiology*, vol. 147, no. 2, pp. 258–264, 2011.

[75] M. Dieterlen, F. W. Mohr, H. Reichenspurner, and M. J. Barten, "Clinical value of flow cytometric assessment of dendritic cell subsets in peripheral blood after solid organ transplantation," *Cytometry A*, vol. 87, no. 5, pp. 377–379, 2015.

[76] P. Athanassopoulos, L. M. B. Vaessen, A. H. M. M. Balk et al., "Impaired circulating dendritic cell reconstitution identifies rejecting recipients after clinical heart transplantation independent of rejection therapy," *European Journal of Cardio-Thoracic Surgery*, vol. 27, no. 5, pp. 783–789, 2005.

[77] P. Athanassopoulos, L. M. B. Vaessen, A. P. W. M. Maat et al., "Preferential depletion of blood myeloid dendritic cells during acute cardiac allograft rejection under controlled immunosuppression," *American Journal of Transplantation*, vol. 5, no. 4, pp. 810–820, 2005.

[78] C. S. K. Ho, J. A. López, S. Vuckovic, C. M. Pyke, R. L. Hockey, and D. N. J. Hart, "Surgical and physical stress increases circulating blood dendritic cell counts independently of monocyte counts," *Blood*, vol. 98, no. 1, pp. 140–145, 2001.

[79] F. S. Dhabhar and B. S. McEwen, "Enhancing versus suppressive effects of stress hormones on skin immune function," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 3, pp. 1059–1064, 1999.

[80] P. Monti, P. Allavena, V. Di Carlo, and L. Piemonti, "Effects of anti-lymphocytes and anti-thymocytes globulin on human dendritic cells," *International Immunopharmacology*, vol. 3, no. 2, pp. 189–196, 2003.

[81] T. Luft, M. Jefford, P. Luetjens et al., "Functionally distinct dendritic cell (DC) populations induced by physiologic stimuli: prostaglandin E_2 regulates the migratory capacity of specific DC subsets," *Blood*, vol. 100, no. 4, pp. 1362–1372, 2002.

[82] E. Scandella, Y. Men, D. F. Legler et al., "CCL19/CCL21-triggered signal transduction and migration of dendritic cells requires prostaglandin E2," *Blood*, vol. 103, no. 5, pp. 1595–1601, 2004.

[83] G. J. Randolph, "Dendritic cell migration to lymph nodes: cytokines, chemokines, and lipid mediators," *Seminars in Immunology*, vol. 13, no. 5, pp. 267–274, 2001.

[84] G. Szabo, C. Gavala, and P. Mandrekar, "Tacrolimus and cyclosporine A inhibit allostimulatory capacity and cytokine production of human myeloid dendritic cells," *Journal of Investigative Medicine*, vol. 49, no. 5, pp. 442–449, 2001.

[85] P.-H. Chiang, L. Wang, C. A. Bonham et al., "Mechanistic insights into impaired dendritic cell function by rapamycin: inhibition of Jak2/Stat4 signaling pathway," *The Journal of Immunology*, vol. 172, no. 3, pp. 1355–1363, 2004.

[86] C. Lagaraine and Y. Lebranchu, "Effects of immunosuppressive drugs on dendritic cells and tolerance induction," *Transplantation*, vol. 75, no. 9, pp. 37S–42S, 2003.

[87] M. Moser, T. De Smedt, T. Sornasse et al., "Glucocorticoids down-regulate dendritic cell function in vitro and in vivo," *European Journal of Immunology*, vol. 25, no. 10, pp. 2818–2824, 1995.

[88] P. P. C. Boor, H. J. Metselaar, S. Mancham, H. W. Tilanus, J. G. Kusters, and J. Kwekkeboom, "Prednisolone suppresses the function and promotes apoptosis of plasmacytoid dendritic cells," *American Journal of Transplantation*, vol. 6, no. 10, pp. 2332–2341, 2006.

[89] M. Shodell and F. P. Siegal, "Corticosteroids depress IFN-α-producing plasmacytoid dendritic cells in human blood," *Journal of Allergy and Clinical Immunology*, vol. 108, no. 3, pp. 446–448, 2001.

[90] G. Raimondi and A. W. Thomson, "Dendritic cells, tolerance and therapy of organ allograft rejection," *Contributions to Nephrology*, vol. 146, pp. 105–120, 2005.

[91] M. Abe and A. W. Thomson, "Influence of immunosuppressive drugs on dendritic cells," *Transplant Immunology*, vol. 11, no. 3-4, pp. 357–365, 2003.

[92] K. L. Womer, R. Peng, P. R. Patton et al., "The effects of renal transplantation on peripheral blood dendritic cells," *Clinical Transplantation*, vol. 19, no. 5, pp. 659–667, 2005.

[93] V. Daniel, "Dendritic cells and immunosuppression after organ transplantation," *LaboratoriumsMedizin*, vol. 32, no. 3, pp. 131–139, 2008.

[94] K. John, M.-T. Dieterlen, A. Tarnok et al., "Role of dendritic cells in the context of acute cellular rejection: comparison between tacrolimus- or cyclosporine A-treated heart transplanted recipients," *Cytometry B—Clinical Cytometry*, vol. 86, no. 5, pp. 362–367, 2014.

[95] M. J. Barten, J. Garbade, H. B. Bittner et al., "Affects of immuno suppression on circulating dendritic cells: an adjunct to therapeutic drug monitoring after heart transplantation," *International Immunopharmacology*, vol. 6, no. 13-14, pp. 2011–2017, 2006.

[96] C. van Kooten, G. Lombardi, K. A. Gelderman et al., "Dendritic cells as a tool to induce transplantation tolerance: obstacles and opportunities," *Transplantation*, vol. 91, no. 1, pp. 2–7, 2011.

[97] U. Švajger and P. Rožman, "Tolerogenic dendritic cells: molecular and cellular mechanisms in transplantation," *Journal of Leukocyte Biology*, vol. 95, no. 1, pp. 53–69, 2014.

[98] A. Moreau, E. Varey, L. Bouchet-Delbos, and M. C. Cuturi, "Cell therapy using tolerogenic dendritic cells in transplantation," *Transplantation Research*, vol. 1, no. 1, article 13, 2012.

[99] A. Lamioni, F. Parisi, G. Isacchi et al., "The immunological effects of extracorporeal photopheresis unraveled: induction of tolerogenic dendritic cells in vitro and regulatory T cells in vivo," *Transplantation*, vol. 79, no. 7, pp. 846–850, 2005.

[100] M.-T. Dieterlen, H. B. Bittner, A. Pierzchalski, S. Dhein, F. W. Mohr, and M. J. Barten, "Immunological monitoring of extracorporeal photopheresis after heart transplantation," *Clinical and Experimental Immunology*, vol. 176, no. 1, pp. 120–128, 2014.

[101] M. Abe, Z. Wang, A. de Creus, and A. W. Thomson, "Plasmacytoid dendritic cell precursors induce allogeneic T-cell hyporesponsiveness and prolong heart graft survival," *American Journal of Transplantation*, vol. 5, no. 8, pp. 1808–1819, 2005.

[102] P. Björck, P. T. H. Coates, Z. Wang, F. J. Duncan, and A. W. Thomson, "Promotion of long-term heart allograft survival by combination of mobilized donor plasmacytoid dendritic cells and anti-CD154 monoclonal antibody," *Journal of Heart and Lung Transplantation*, vol. 24, no. 8, pp. 1118–1120, 2005.

[103] H. Li and B. Shi, "Tolerogenic dendritic cells and their applications in transplantation," *Cellular and Molecular Immunology*, vol. 12, no. 1, pp. 24–30, 2015.

[104] S. Gregori, D. Tomasoni, V. Pacciani et al., "Differentiation of type 1 T regulatory cells (Tr1) by tolerogenic DC-10 requires the IL-10-dependent ILT4/HLA-G pathway," *Blood*, vol. 116, no. 6, pp. 935–944, 2010.

[105] L. J. Young, N. S. Wilson, P. Schnorrer et al., "Differential MHC class II synthesis and ubiquitination confers distinct antigen-presenting properties on conventional and plasmacytoid dendritic cells," *Nature Immunology*, vol. 9, no. 11, pp. 1244–1252, 2008.

[106] O. H. Gerstner, W. Laffers, and A. Tárnok, "Clinical applications of slide-based cytometry—an update," *Journal of Biophotonics*, vol. 2, no. 8-9, pp. 463–469, 2009.

[107] S. M. Cherian, Y. V. Bobryshev, S. J. Inder, A. Y. Wang, R. S. A. Lord, and A. E. Farnsworth, "Dendritic cells in aortocoronary saphenous vein bypass grafts," *Heart Lung and Circulation*, vol. 9, no. 1, pp. 39–42, 2000.

Hindawi Publishing Corporation
Journal of Immunology Research
Volume 2016, Article ID 6269157, 15 pages
http://dx.doi.org/10.1155/2016/6269157

Review Article

Breakdown of Immune Tolerance in Systemic Lupus Erythematosus by Dendritic Cells

Xiaofeng Liao, Alec M. Reihl, and Xin M. Luo

Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

Correspondence should be addressed to Xin M. Luo; xinluo@vt.edu

Received 23 October 2015; Revised 15 January 2016; Accepted 7 February 2016

Academic Editor: Christine Trumpfheller

Dendritic cells (DC) play an important role in the pathogenesis of systemic lupus erythematosus (SLE), an autoimmune disease with multiple tissue manifestations. In this review, we summarize recent studies on the roles of conventional DC and plasmacytoid DC in the development of both murine lupus and human SLE. In the past decade, studies using selective DC depletions have demonstrated critical roles of DC in lupus progression. Comprehensive *in vitro* and *in vivo* studies suggest activation of DC by self-antigens in lupus pathogenesis, followed by breakdown of immune tolerance to self. Potential treatment strategies targeting DC have been developed. However, many questions remain regarding the mechanisms by which DC modulate lupus pathogenesis that require further investigations.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that causes damage of multiple organs [1]. Disease activity and stages can be generally divided into three patterns—the remitting relapsing pattern, chronically active disease, and long quiescence—based on various clinical manifestations that include, but are not limited to, skin rash, arthritis, nephritis, hematological disorders, and neurological disorders [2]. During SLE pathogenesis, autoreactive T cells are activated which in turn activate autoreactive B cells to produce high affinity autoantibodies against self-antigens [3]. Immune complexes (ICs) formed by aggregation of autoantibodies and self-antigens circulate in the blood and eventually deposit in peripheral tissues where the complement system is activated, ultimately inducing the release of signals that further recruit and activate autoreactive cells to feed forward a vicious cycle of chronic inflammation. Different innate and adaptive immune cell populations, including monocytes/macrophages, neutrophils, dendritic cells (DC), and lymphocytes, are recruited into peripheral tissues following the inflammatory signals to amplify inflammation and cause tissue damage [1, 4–6].

DC were discovered as the professional antigen-presenting cells (APC) with a primary function of priming naïve T cell activation [7]. Since their discovery, our understanding of how DC contribute to immune responses has much expanded, and DC have been divided into many subpopulations with distinct phenotypes and functions [8]. Two main subpopulations are classical DC (cDC) and plasmacytoid DC (pDC). DC are developed from a series of dedicated DC progenitors [8]. Common dendritic cell progenitors (CDP) originated from macrophage dendritic cell progenitors (MDP) are the first dedicated DC progenitors that can differentiate into pre-cDC and pDC in bone marrow. Pre-cDC then migrate into lymphoid and nonlymphoid tissues to differentiate into cDC. Monocytes originated from MDP can also differentiate into cDC in lymphoid and nonlymphoid tissues [8]. Murine cDC is characterized by high expression of CD11c and MHC-II on surface, while human cDC also express nonoverlapping makers CD1c (blood dendritic cell antigen 1 or BDCA1) or CD141 (BDCA3) on different subsets besides CD11c and MHC-II. Different from cDC, murine pDCs express low level CD11c and MHC-II but are positive for B220 and Siglec-H on surface, and human pDCs are defined by the expression of MHC-II, BDCA2, and BDCA4.

Functionally, cDC are professional APC that prime naïve T cells upon antigen uptake and maturation induced by appropriate maturation signals (e.g., upon TLR ligation). Mature cDC start to prime naïve T cells with the interaction between MHC-II-peptide complex on cDC and T cell receptor on T cells. The ligation of costimulatory molecules, with CD80 and CD86 on cDC and CD28 on T cells, further mutually activates cDC and T cells. Finally the cytokines secreted by cDC induce the differentiation of naïve T cells into different effector helper T cell subsets. pDCs, on the other hand, are professional interferon α (IFNα) producing cells that, through producing a high level of IFNα, activate multiple immune cell populations that express type I IFN receptor (IFNAR) [9]. Interestingly, pDC can also upregulate MHC-II upon activation and act like cDC to activate T cells [10].

Both cDC and pDC are important for immune tolerance to self [8]. Immature cDC when presenting self-antigens in the absence of maturation stimuli express low level MHC-II on the surface and induce immune tolerance to self. Upon activation by maturation stimuli, however, cDC mature with upregulation of MHC-II and activation markers (CD40, CD80, CD86, PD-L1, PD-L2, etc.) to facilitate inflammation. For pDCs, while their primary function is to control infections, pDCs in thymus are involved in the negative selection to maintain the central tolerance. Not surprisingly, studies have shown that both cDC and pDC play important roles in the development of autoimmune diseases, such as SLE [11].

Peripheral blood mononuclear cells (PBMC) from SLE patients can be used to study *in vitro* DC responses. Whilst important, information obtained from blood cells is limited. To this end, lupus-prone mouse models that develop lupus-like symptoms spontaneously or artificially can be used to better understand DC-mediated mechanisms of lupus progression under both *in vivo* and *in vitro* conditions. In this review, we summarize recent results obtained from studies of SLE patients and lupus-prone mice on the roles of cDC and pDC in lupus development.

2. *In Vivo* DC Depletion Studies: Indication of DC Involvement in Lupus

A direct strategy to study whether a cell population is critical for the development of a disease is to deplete the population *in vivo*. Depletion of DC in wild-type mice and lupus-prone mice shows differential contributions of DC to immune homeostasis, with a tolerogenic role of DC in wild-type mice versus an immunogenic role of DC in lupus-prone mice. In wild-type mice, constitutive depletion of $CD11c^{high}$ cDC showed normal development of regulatory T (Treg) cells and normal negative selection of $CD4^+$ T cells in the thymus without an autoimmune response [12]. Constitutive depletion of both cDC and pDC in wild-type mice, however, led to increased autoimmune inflammation with elevated autoantibodies, increased IFNγ/IL-17-secreting T cells in peripheral tissues, and abnormal negative selection of $CD4^+$ T cells in the thymus [13]. This suggests that pDC, or the combination of pDC and cDC, may contribute to immune tolerance to self. Interestingly, regardless of the presence of pDC, the absence of cDC consistently resulted in dramatic expansion of myeloid cells, particularly neutrophils and macrophages [12, 13].

In MRL/lpr lupus-prone mice, constitutive depletion of cDC and pDC did not influence the negative selection of T cells in the thymus. However, it led to fewer splenic Treg cells and less CD25 expression on the surface of these cells, suggesting compromised immune tolerance in MRL/lpr mice in the absence of DC [14]. Importantly, even though myeloid cells expanded dramatically as in wild-type mice [12, 13], glomerulonephritis and dermatitis were significantly reduced with DC depletion in MRL/lpr mice, which was accompanied by a significant decrease of the proliferation of total T cells and IFNγ-producing effector T cells. The lack of DC also led to significantly fewer plasmablasts and impaired autoantibody production and class switching to IgG, the primary autoantibody isotype in lupus [14]. These results demonstrate a critical role of DC in promoting lupus-like disease in MRL/lpr mice. Interestingly, the initiation of T cell activation in lupus may be DC-independent, as the ratio of naïve to activated T cells in the spleen did not change with DC depletion. It appears that autoreactive B cells, instead of DC, initiate the activation of autoreactive T cells through antigen presentation in MRL/lpr mice [15]. These data suggest that although DC can maintain immune tolerance to self in wild-type mice, overall their functions have switched to promoting autoimmune responses in lupus-prone mice.

For pDC, early transient depletion of these cells from BXSB (Yaa) lupus-prone mouse model inhibited type I IFN signature, reduced T and B cell activation, decreased autoantibody production, and improved lupus nephritis [16]. While pDC reappeared later on, the effect of early depletion was sustained, suggesting that pDCs contribute to lupus disease at the initiation stage. This observation has been confirmed by another study using B6.Nba2 lupus-prone mice [17].

These depletion studies indicate the importance of cDC and pDC in the development of lupus. Therefore, we will next summarize in detail how cDC and pDC, respectively, break down immune tolerance to self and facilitate lupus progression.

3. Breakdown of Immune Tolerance to Self in SLE by cDC

3.1. Changes of cDC Number and Phenotype in Lupus. Changes of cell number and phenotype may reflect changes of the cells' activation status and/or their dynamic trafficking into different tissues. Studies on the changes of cDC number and phenotype in lupus will help us understand whether cDC are activated and where they function to break down self immune tolerance. In SLE patients, a general sense is that cDC number and frequency in the blood are lower with higher disease activity [18–23]. The decrease of blood cDC may be due to increased migration of cDC into peripheral tissues. For example, more cDC were found to infiltrate the tubulointerstitial region in the kidney biopsy of SLE patients with proliferative or active nephritis than the healthy control (HC) or patients with nonproliferative nephritis, and the increase in renal infiltration was accompanied by a decrease

of cDC number in the peripheral blood [20, 24]. Murine cDC, particularly those expressing CD11b, also accumulated in the kidney of various types of lupus-prone mice as lupus nephritis progressed [25–27]. In addition, we and others showed increased cDC accumulation in the spleen and lymph nodes of lupus-prone mice [28–32]. How cDC infiltrated inflamed tissues is unclear, but studies have shown that chemokine receptors chemR23 and CCR7 may be important for cDC migration into the kidney and secondary immune tissues, respectively [32–35]. Renal expression of chemerin—the chemokine ligand of chemR23—and increased chemR23$^+$ DC in the kidney of SLE patients suggest chemerin-dependent migration of cDC into inflamed kidney in lupus [34]. CCR7, on the other hand, mediates migration of cDC to lymph nodes. Upon IFNα priming and lipopolysaccharide (LPS) stimulation, monocyte-derived cDC (moDC) from SLE patients expressed a significantly higher level of CCR7 [35]. Besides IFNα and LPS, ICs can also induce the migration of moDC towards CCR7 ligands both *in vitro* and *in vivo* [32].

The phenotype of cDC is different between tolerogenic cDC, which suppress inflammation, and immunogenic cDC that stimulate inflammation. cDC in the blood of SLE patients or secondary immune tissues of lupus-prone mice have been shown to exhibit elevated expression of CD40, CD80, CD86, PD-L1, and PD-L2, suggesting that cDC in lupus may be activated and immunogenic [18, 36–38]. However, *in vitro* studies using moDC from SLE patients or lupus-prone mice have shown inconsistent results regarding the activation phenotype of cDC [18, 36, 39–41]. Some showed higher activation state of moDC and enhanced T cell activation with lupus, while others showed either comparable activities or reduced moDC and T cell activation. The inconsistency may be due to different methods used for moDC differentiation, maturation, and activation, as different amounts of granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-4 were used to generate immature moDC, and different stimuli (e.g., LPS, TNFα, CpG, or IFNα) were used to mature or activate moDC in different studies.

3.2. MoDC in Lupus. Monocytes can differentiate into cDC under both steady state and inflammatory state *in vivo* [8]. GM-CSF and IL-4 can also induce moDC *in vitro* [42]. However, whether monocytes are a precursor of cDC in lupus is still an open question. Monocytes incubated with sera from SLE patients could differentiate into cDC, but the differentiation depended on the presence of IFNα in the serum [43]. Later studies also showed that IgG-containing ICs in the serum, tumor necrosis factor (TNF) receptor I on monocytes, and the interaction between monocytes and T cells are all important for the differentiation of monocytes into cDC in lupus [35, 43, 44]. Regarding the function of differentiated moDC, only those generated in the presence of SLE sera, rather than moDC generated by IFNα/GM-CSF alone, could promote differentiation of IgG- and IgA-producing plasmablasts from B cells. This suggests that factors other than IFNα in the SLE patient sera affect the function of moDC in lupus [45].

3.3. Regulation of cDC Activation in Lupus. As discussed earlier, activated cDC accumulate in lymphoid and nonlymphoid tissues during lupus progression. It is important to understand how they are activated in the context of lupus. *In vitro* studies suggest that self-DNA and/or self-RNA containing antigens could activate cDC [46–48]. *In vitro* generated moDC from both healthy human PBMC and wild-type mouse bone marrow can be activated by necrotic or apoptotic cell particles containing self-DNA and self-RNA to produce inflammatory cytokines (IL-6, TNFα), upregulate MHC-II and costimulatory molecules (CD40, CD80, and CD86), and activate allogeneic T cells that in turn produce IL-2, IFNγ, and IL-17. It has been demonstrated that cDC generated *in vitro* or isolated directly from human or mouse could be activated by DNA- and RNA-containing self-antigens through the signaling of toll-like receptor (TLR)9 and TLR7/8, respectively [49–54]. However, it is still unclear whether cDC can be activated by nucleic acid-containing self-antigens *in vivo*, because natural IgM antibodies and complement C1q-opsonized apoptotic particles, both present *in vivo* but not necessarily in *in vitro* experiments, have the ability to suppress cDC activation [55–57]. The suppression of p38 MAPK phosphorylation by MAPK phosphatase-1 appears to be important for cDC tolerance induced by natural IgM [56].

Studies using gene knockouts in mice have shown that TLR7, MyD88, and interferon regulatory factor (IRF)5 are important for cDC activation in lupus, and TLR8, A20, Lyn, B lymphocyte-induced maturation protein-1 (Blimp1), and Bim can downregulate cDC activation [58–65]. While TLR7 promotes cDC activation in lupus, TLR8 downregulates TLR7 expression and TLR7-dependent cDC activation [58]. IRF5-deficient cDC exhibited a reduced ability to produce TNFα, IL-6, and IL-10 in lupus-prone mice [61]. DC-specific deficiency of A20, Lyn, or Blimp1 led to lupus-like disease in mice [60, 62–64]. cDC isolated from Bim$^{-/-}$ mice compared to wild-type mice induced higher T cell proliferation *in vitro*, and autoantibodies can be generated in non-lupus-prone mice upon transfer of Bim-deficient cDC [65]. The role of MyD88 in lupus cDC is debated. One study using MyD88-deficient MRL/lpr mice showed no obvious change of lupus nephritis [59], while another study using DC-specific MyD88 and Lyn double-deficient mice showed attenuated lupus disease compared to DC-specific Lyn-deficient mice [60]. Interestingly, polymorphisms within *TLR7*, *IRF5*, *TLR8*, *A20*, *Lyn*, and *Blimp1* gene loci have all been shown to be associated with SLE [66–70].

Activation of cDC can be regulated by several additional factors according to studies of SLE patient samples. Expression of immunoglobulin-like transcript (ILT)3, an inhibitory receptor, was found to be decreased on circulating cDC of SLE patients, and the decrease was correlated with higher levels of proinflammatory cytokines (type I IFN, TNFα) in the plasma of these patients [71]. Not surprisingly, SLE-susceptible single nucleotide polymorphisms were identified in the *ILT3* gene locus. Sex hormones may also affect the activation of cDC. In a minichromosome maintenance protein (MCM)6 dependent manner, 17beta-estradiol, a female hormone, could induce upregulation of CD40 on *in vitro*-generated moDC

that in turn increased T cell activation [72]. cDC purified from SLE patients compared to HC expressed a higher level of MCM6, and MCM6 expression was positively correlated with the level of 17beta-estradiol in the sera of SLE patients [72]. Moreover, cDC activation is affected by complement C1q, although the effect of complement C1q on cDC is still unclear. One study showed that immobilized C1q coated on plates induced maturation of immature moDC differentiated *in vitro* from healthy PBMC by GM-CSF/IL-4 [73]. Mature moDC, compared to immature moDC, had increased production of IL-12, TNFα, and IL10 and enhanced T cell proliferation and secretion of IFNγ. However, another study showed that, when immobilized C1q was added concurrently with GM-CSF/IL-4 during moDC differentiation from PBMC, moDC stayed at immature state [74]. Upon LPS or LPS/IFNγ stimulation, these moDC did mature, but they produced less IL-12, TNFα, and IL-6 but more IL-10 [74]. Mature cDC generated by LPS or LPS/IFNγ also had reduced ability to activate T cells. The timing of C1q addition appears to be important, and further studies are required to uncover the roles of C1q in regulating cDC maturation and activation.

Apoptosis of activated cDC is important for immune tolerance to self. Under normal condition, activated cDC undergo apoptosis through either Fas-dependent or mitochondria-dependent pathways, the latter by interacting with activated Treg cells that express lymphocyte-activation gene (LAG)3 [75, 76]. DC-specific deficiency in either Fas-dependent or Fas-independent apoptosis in mice could induce lupus-like symptoms, suggesting that abnormal accumulation of activated cDC may contribute to breakdown of self-tolerance and lupus development [75–77].

3.4. Activation of T Cells and B Cells by cDC in Lupus. Upon activation by self-antigens, cDC can promote lupus development by interacting with T cells and B cells. While *in vivo* studies of how cDC affect autoreactive T cells are still lacking, *in vitro* evidence suggests that moDC derived from the bone marrow of lupus mice or from PBMC of SLE patients, upon activation, can promote T cell activation and hamper Treg response [39, 52, 78–80]. It is demonstrated in both mouse and human cell studies that moDC activated by apoptotic cells or cytosolic dsDNA could induce the activation of T cells, including that of autoreactive T cells [52, 79]. In addition, compared to bone marrow-derived macrophages, bone marrow-derived cDC (BMDC) from lupus-prone mice possessed higher ability to activate autoreactive T cells, suggesting that cDC rather than macrophages are the APC for autoreactive T cell activation [78]. Moreover, *in vitro* generated tolerogenic BMDC from SLE patients were less capable of generating Treg cells *in vitro* than HC BMDC [80]. Furthermore, LPS-activated BMDC from lupus-prone mice suppressed Treg function by producing more IL-6, which indirectly promoted proliferation of $CD4^+$ T cells [39].

Several studies using *in vitro* systems have indicated possible roles of cDC in promoting autoreactive B cell activation [45, 52, 81–83]. A couple of them have shown that GM-CSF/IL-4-induced BMDC from B6.Sle1.Sle2.Sle3 lupus-prone mice, compared to BMDC from B6 mice, promoted better B cells proliferation and IgM/IgG production in *in vitro* coculture system upon anti-CD40 ligation [81, 82]. The enhancement was partially dependent on elevated IL-6 and IFNγ produced by activated BMDC. In addition, upon i.p. injection of ICs, splenic $CD11c^+$ DC from B6.Sle1.Sle2.Sle3 mice produced more IL-6 and IFNγ than those from B6 mice. In human cell studies, moDC derived from healthy PBMC *in vitro* activated by either the sera from SLE patients or cytosolic dsDNA promoted B cell antibody class switch to IgG and IgA [45, 52]. Contradictory to these observations, however, one study showed that BMDC from several lupus-prone mouse models, when activated by LPS, possessed reduced IL-6-producing ability compared to BMDC from B6 mice [83]. Due to the decrease of IL-6 production, LPS-activated BMDC from MRL/lpr mice failed to suppress autoreactive IgM production by B cells. The discrepancy may have been due to different lupus-prone mouse models used or different activation methods (anti-CD40 versus LPS), although another study has shown that LPS could increase IL-6 production from BMDC of B6.Sle1.Sle2.Sle3 mice [39].

Besides activating T cells and B cells, cDC may also promote lupus development by producing high-mobility group box 1 (HMGB1) protein that not only binds nucleosomes to facilitate activation of cDC as a positive feedback but also enhances IFNα production by pDC, the latter of which will be discussed below [46, 49, 84].

3.5. Potential Treatment Strategies of Lupus by Targeting cDC. Since cDC can promote lupus development, they are a potential target for the development of new drugs against lupus. To target innate immune cells such as cDC, nanogel-based immunosuppressive drugs have been tested in lupus-prone mice that led to prolonged survival and reduced lupus nephritis [85, 86]. The lipid coating of nanogel enables better uptake of the drug by cDC, thus increasing the amount of immunosuppressive drug inside the cells. In addition, *in vitro* studies have shown that BMDC incubated with immunosuppressive drug-containing nanogel had lower production of inflammatory cytokines compared to cells incubated with free drug. The ability of pDC to produce IFNα was also suppressed, with less IFNα produced in the presence of nanogel [85]. It appears that cDC-targeted therapies may benefit from nanogel-based delivery with minimal side effects.

Efforts have been made to induce the generation of tolerogenic cDC to ameliorate lupus. Several studies have shown that tolerogenic cDC generated by transgenic method or induced *in vitro* can rebuild immune tolerance to self after adoptive transfer to lupus-prone mice [87–89]. Tolerogenic cDC can also be induced from PBMC of SLE patients *in vitro* to suppress T cell activation [18, 90].

4. Breakdown of Immune Tolerance to Self in SLE by pDC

4.1. Changes of pDC Number and Phenotype in Lupus. pDCs play an important role in lupus development in addition to cDC. Human studies of pDC frequency and number in the blood of SLE patients have shown inconsistent results [19–21, 91–95]. The inconsistency may reflect the dynamic

change of cell number and migration of pDC corresponding to different disease stages and/or treatments. The decrease of pDC in the circulation of some SLE patients may indicate increased migration of the cells into peripheral tissues. Notably, increased infiltration of pDC to the kidney of SLE patients has been confirmed by several studies [20, 24, 95], although the location of the infiltrate is still a matter of debate. It has been suggested that pDC may use IL-18 receptor and chemR23 to migrate into the inflamed kidney that expresses IL-18 and chemerin, respectively [33, 34, 95]. In mice, however, one study showed no change of pDC in the kidney as lupus progressed [27]. pDC can also accumulate in the skin of SLE patients and lupus-prone mice [96, 97]. In MRL/lpr lupus-prone mice, UVB irradiation induces skin infiltration of pDC, while IFNα response in the skin has been shown to be positively correlated with the level of chemerin that can attract pDC through chemR23 [97].

Conversely, the increase of pDC in the circulation of some SLE patients may be due to increased generation and emigration of pDC from the bone marrow. Our study using MRL/lpr mice demonstrated that the number of pDC was increased in the bone marrow compared to MRL control mice [28]. A higher percentage of pDC was also found in the bone marrow of SLE patients compared to HC [98]. It is worth noting that phenotypic identification of pDC varies from one study to another and that the surface markers used to define pDC in healthy individuals may not be appropriate under the disease environment [99].

However, we and others have consistently observed the expansion of pDC in secondary immune tissues during lupus progression. We have found that pDC are increased in the MLN of young MRL/lpr mice compared to age-matched MRL controls [28]. Others using NZB/W F1 mice and NZM2328 mice have found similar results in MLN and renal lymph nodes [38, 100]. pDCs also accumulate in the spleen of lupus-prone mice, particularly in the marginal zone (MZ) of the spleen [30, 38, 82, 101, 102]. The increase of pDC in secondary lymph tissues on one hand may be caused by inflammation-induced migration and/or self-expansion *in situ*, as will be discussed later. On the other hand, pDCs appear to be able to survive better in lupus [102–104], as their expression of antiapoptotic Bcl-2 was found to be increased [102]. Survival signal in pDC from both humans and lupus-prone mice is activated by TLR7/9-induced NFκB pathway [103, 105]. pDCs in lupus are constantly stimulated by TLR7/9 ligands, which are known to suppress miR-29b and miR-29c, allowing for upregulation of the target of these microRNAs, including Bcl-2 [104].

Many functional markers expressed on pDC are altered in SLE patients and lupus-prone mice. The expression of three inhibitory receptors, BDCA2, leukocyte-associated immunoglobulin-like receptor 1 (LAIR-1), and ILT3, on human pDC is reduced in SLE patients compared to HC [94, 106, 107]. On the contrary, MHC-II and costimulatory molecules are increased on pDC of both SLE patients and lupus-prone mice, suggesting an increased ability to present self-antigens and activate autoreactive T cells [28, 37, 38, 101, 108, 109].

4.2. Critical Roles of IFNα in Lupus Development. One major function of pDC in immune responses against foreign pathogens is to produce a large amount of type I IFN. Many studies have shown that type I IFN, particularly IFNα, is critical for lupus development. It is well known that SLE patients have elevated serum IFNα level that is positively correlated with disease severity [43]. Administration of IFNα into humans for antivirus or antitumor treatment, or into preautoimmune lupus-prone mice, can induce or accelerate lupus-like symptoms [110–112]. Deficiency of the receptor of type I IFN and IFNAR in several lupus-prone mouse models resulted in ameliorated lupus symptoms [100, 113, 114]. Interestingly, anti-IFNAR treatment transiently ameliorated lupus disease in MRL/lpr mice, but constitutive depletion of IFNAR in the same model deteriorated lupus symptoms [115, 116]. IFNβ deficiency in BXSB mice failed to modify lupus progression, indicating that the IFNα subtype is the principal type I IFN important for lupus development [116]. Recent studies have shown that by either depleting pDC or abrogating IFNα production of pDC, lupus disease is reduced [16, 17, 117]. However, only the depletion of pDC or blockade of IFNα signaling at early stage of disease could prevent lupus development [17, 116]. Together, these studies suggest that through secreting IFNα, pDC may play a critical role in the development of lupus disease at the early initiation stage.

Many types of leukocytes can express IFNAR on the surface and respond to IFNα, including monocytes, cDC, pDC, T cells, and B cells [116]. Sera from SLE patients can induce normal monocytes to differentiate into cDC in an IFNα-dependent manner [43]. Differentiated cDC can subsequently activate both allogeneic and autologous $CD4^+$ T cells. IFNα can also expand splenic cDC, particularly $CD11b^+CX3CR1^+$ cDC, that may have been derived from monocytes [30]. In addition, IFNα is able to precondition the immunogenic status of monocytes. Without IFNα priming, monocytes incubated with RNA-containing ICs from SLE patients failed to upregulate activation markers [118]. The same phenomenon was observed for moDC differentiated by apoptotic blebs or apoptotic cells, where IFNα priming enabled these moDC, which were tolerogenic without IFNα, to activate T cells [119, 120]. The molecular mechanism of how IFNα activates monocytes is still unclear, but studies have shown increased expression of two IFNα inducible genes, Ifi202 in bone marrow-derived DC from lupus-prone mice and Ifit4 in monocytes from SLE patients [121, 122]. Overexpression of these genes can activate normal moDC with enhanced IL-12 production, which promotes Th1 differentiation. Besides activation, IFNα also affects the migration of moDC. IFNα/GM-CSF-induced rather than IL-4/GM-CSF-induced moDC from healthy human PBMC can upregulate MMP-9 and migrate towards CCL5 and CCL3 that are expressed in inflamed tissues [123].

IFNα also influences pDC themselves as well as non-monocyte-derived cDC. In lupus-prone mice, IFNα-dependent expansion of splenic pDC has been documented [30]. With IFNAR-I deficiency, both cell number and surface activation markers of splenic pDC were reduced [100]. In the case for non-monocyte-derived cDC, studies of IFNAR-I-deficient NZM2328 mice have shown reduced splenic $CD8^+$

and $CD8^-$ cDC with decreased activation markers [100]. IL-12- and TNFα-producing ability of $CD8^+$ cDC was also reduced in the absence of IFNAR-I [100].

Regarding T cells, an *in vitro* study showed that normal cDC primed by IFNα could promote naïve T cells to differentiate into Th1/Th17 T cells [124]. However, if IFNα was constantly present in the cDC-T cell coculture system, it had a suppressive effect for Th1/Th17 differentiation. IFNα can also promote inflammatory T cell function by inducing the migration of effector T cells into inflamed tissues in a CXCR3-dependent manner [125].

Studies on lupus-prone mouse models have shown that IFNα-producing pDC can directly influence autoreactive B cell response. In BXD2 lupus-prone mice, it was demonstrated that the accumulation of activated pDC in the MZ of spleen resulted in the upregulation of CD86 on MZ B cells, which was important for germinal center (GC) formation and autoantibody production [126]. In addition, MZ B cells increased their migration into the follicular region in response to IFNα produced by the accumulated pDC. Such migration of B cells reduced the interaction with MZ macrophages, causing the macrophages to decrease in number in the MZ [127]. This would compromise clearance of apoptotic cells in the spleen of lupus-prone mice and promote exposure of autoantigens to DC, autoreactive T cells, and B cells.

4.3. Regulation of IFNα Production from pDC in Lupus. Due to the critical role of IFNα in lupus development, how pDC are activated to produce IFNα in lupus has been studied. pDCs produce a large amount of IFNα upon TLR7 and TLR9 stimulation by bacterial or viral nucleic acids [8]. Thus, infections could be a trigger of IFNα production by pDC in lupus. One study showed that Epstein-Barr virus (EBV) infection was associated with lupus [128]. In addition, nucleic acid self-antigens and/or nucleic acid-containing ICs are another potential inducer of TLR7/9-dependent IFNα production by pDC in lupus [128]. Nucleic acid self-antigens derived from apoptotic or necrotic cells are increased significantly in SLE patients and lupus-prone mice compared to respective controls [1]. When the sera of SLE patients were mixed with healthy PBMC, more IFNα production was induced from pDC [129]. The patient sera contained ICs formed between IgG and apoptotic cells, which were found to activate pDC to produce IFNα through TLR7/9 [53, 130–133]. Interestingly, IgG alone or ICs with nucleic acid digestion failed to induce IFNα production by normal pDC, suggesting a critical role of TLR7/9 stimulation by nucleic acids within the ICs. However, DNA/RNA alone or nucleic acid-containing ICs in the presence of FcγRAII blockade also could not trigger pDC to produce IFNα, indicating that the interaction between IgG in ICs and FcγRAII on pDC is important for IC-induced IFNα production by pDC [130, 133]. Moreover, it has been shown that CpG motif in dsDNA of DNA-containing ICs is required for IFNα production by normal pDC [50].

Nucleic acid self-antigens can also induce IFNα production by pDC in an Fc receptor- (FcR-) independent pathway free from the formation of ICs. LL37, an antimicrobial peptide, has been shown to complex with self-DNA and self-RNA to form nanoscale aggregates that trigger IFNα production by normal pDC in a TLR7/9-dependent manner [54, 134]. Neutrophils from SLE patients possess an increased ability to release neutrophil extracellular traps (NETs), which contain LL37 [108, 135]. When LL37 was digested, NETs were no longer able to induce IFNα production by pDC, suggesting a critical role for this peptide [135]. IFNα in turn can upregulate LL37 and HNP (another antimicrobial peptide) on the surface neutrophils as seen in the blood of SLE pateints [108]. The levels of anti-LL37 and anti-HNP antibodies in the patient sera are also increased, which, when ligated with transmembrane expressed LL37 and HNP, respectively, can trigger the release of NETs by neutrophils. These results suggest that a positive feedback loop between NETs release by neutrophils and IFNα production by pDC may initiate and/or promote lupus development in SLE patients. Interestingly, LL37 has been found to be also important for FcγRIIA-dependent IFNα production from pDC, likely through facilitating the internalization of ICs [135].

Signaling molecules in the TLR7/9 pathway are important for autoantigen-induced IFNα production from pDC. SLC15A4-, MyD88-, IRF8-, or IRF5-deficient lupus-prone mice have shown ameliorated lupus symptoms with reduced IFNα protein level in the serum, decreased IFNα transcript level in pDC, downregulation of type I IFN inducible genes, and suppressed activation of both T cells and B cells [59, 61, 136–138]. In addition, pDC from IRF-5- or IRF7-deficient mice failed to produce IFNα upon stimulation with RNA-containing ICs from the sera of SLE patients [50, 139]. Moreover, interleukin-1 receptor-associated kinase (IRAK)1 and IRAK4 are required for IFNα induction from pDC, as their inhibition abrogates the production of IFNα from healthy pDC stimulated with the sera of SLE patients [140].

The ability of pDC to produce IFNα is also regulated by many other factors that may influence the outcome of lupus development. High-mobility group box (HMGB) proteins, for example, function as universal sentinels for nucleic acid-mediated immune response through both cytosolic receptors and those in endosomes including TLR9 and TLR7 [141]. It has been shown that, compared to CpG-A alone, HMGB1-bound CpG-A could induce higher IFNα and TNFα production by normal pDC [142]. This is due to increased recruitment of MyD88 to TLR9 in the presence of HMGB1. In addition, HMGB1 can facilitate the formation of CpG-TLR9 complexes and retain the complexes in early endosome rather than lysosome, resulting in sustained IFNα production by pDC [49]. Studies on SLE patient samples have shown that the level of HMGB1 in the circulation was positively correlated with the concentration of IFNα [46, 107]. Moreover, the interaction between HMGB1 and receptor for advanced glycation endproducts (RAGE) is required, as PBMC from HC incubated with the sera of SLE patients produce much less IFNα when the interaction is blocked [46, 142].

Amyloid fibrils can also regulate IFNα production from pDC by modulating the trafficking of nucleic acid-TLR complexes. These are stable insoluble aggregates of misfolded protein products with extensive β-sheet structure that can facilitate the maintenance of nucleic acid antigens in early endosomes of pDC [143]. Albeit rare, amyloid fibrils have been

found to be associated with SLE cases and complicate lupus nephritis [144]. Immunization of healthy mice with DNA-containing amyloid fibrils induces lupus-like disease, promoting autoantibody production and lupus nephritis [143].

C-reactive protein (CRP), an acute-phase reactant produced by liver in response to inflammation, can suppress IFNα production from normal pDC by increasing the trafficking to ICs into late endosomes in pDC [132]. Therefore, CRP may be beneficial for lupus disease through inhibiting IFNα production. In SLE patients, the elevation of CRP in response to inflammation is modest and much less than expected, suggesting compromised regulation of IFNα production [145].

Complement C1q is another suppressive factor of IFNα production from pDC. Human individuals with C1q-deficiency can develop SLE [146, 147]. When C1q is added simultaneously, RNA-containing ICs or CpG stimulated less production of IFNα, IL-6, IL-8, and TNFα from PBMC or purified healthy pDC [148]. The suppressive effect of C1q on IFNα production from pDC has been shown to be dependent on the ligation of C1q to LAIR-1 expressed on pDC [149].

Sex hormones may also regulate IFNα production from pDC in SLE patients. One study has shown that that TLR7 agonist induced higher IFNα production by PBMC from healthy women than those from healthy men [150]. In addition, 17beta-estradiol, a female hormone, can increase IFNα production from pDC upon CpG stimulation [151].

pDC may interact with other cell types *in vivo* that affect their ability to produce IFNα. Studies have shown that B cells, platelets, NK cells, and monocytes can differentially influence IFNα production by pDC [152–156]. B cells facilitate IFNα production by normal pDC stimulated with RNA-containing ICs or CpG-A [152]. Interestingly, the mechanisms of B cell involvement are different depending on the stimulation. For RNA-containing ICs, the contact between B cells and pDC through adhesion molecule CD31 is required, while the elevation of CpG-induced IFNα production is B cell contact-independent. The latter may be dependent on an unknown secreted molecule, as the supernatant from CpG-A-stimulated B cell culture could promote IFNα production from pDC. In addition, activated platelets, found to be more abundant in the blood of SLE patients, can promote IFNα production from normal pDC stimulated with nucleic acid-containing ICs through interaction between CD154 on platelets and CD40 on pDC [153]. In lupus-prone mice, depletion of platelets improved, while administration of activated platelets worsened, lupus disease, suggesting the involvement of platelets in lupus development. Moreover, $CD56^{dim}CD16^{+}$ NK cells can promote IFNα production from pDC upon stimulation with RNA-containing ICs in the coculture of pDC and NK cells through both secreted MIP-1β and CD11a-dependent direct contact between the two cell types [154, 155]. NK cells isolated from SLE patients, however, produced less IFNα than NK cells from HC, since most of them were $CD56^{bright}CD16^{-}$ rather than $CD56^{dim}CD16^{+}$ NK cells. Furthermore, $CD14^{+}$ monocytes, contrary to B cell, platelets, and NK cells, can suppress IFNα production from pDC through various mechanisms. It has been shown that upon RNA-containing ICs stimulation, $CD14^{+}$ monocytes produced TNFα, prostaglandin E2, and reactive oxygen species, all of which suppressed IFNα production from normal pDC in coculture [155]. Additionally, monocytes can suppress IFNα production from normal pDC through competitive binding of C1q-coated ICs to reduce internalization of ICs in pDC [156]. Monocytes isolated from SLE patients have less suppressive effect on IFNα production from pDC compared to those isolated from HC [155].

4.4. IFNα-Producing Ability of pDC in Lupus. While the essential role of IFNα-producing pDC in lupus is inarguable, questions remain on whether pDCs are the major IFNα-producing cells during the entire course of lupus progression. It has been demonstrated in several studies that PBMC or pDC purified from PBMC of SLE patients produced much less IFNα upon TLR9-ligand stimulation compared to HC [93, 157–159]. Similar results have been obtained from lupus-prone mice [101]. We have shown in our recent study that pDC isolated from older MRL/lpr mice in the late stage of lupus development produced significantly less IFNα upon CpG stimulation *in vitro* compared to pDC purified from younger mice in the early stage [109]. The reduced IFNα-producing ability may be due to continuous exposure to nucleic acid self-antigens, as pDC from HC produced much less IFNα after repeated stimulation with CpG or DNA-containing ICs [159]. Notably, one study showed comparable IFNα production between pDC from SLE patients versus healthy individuals [160]. In their study, however, IL-3 was added in cell culture medium, which may have enhanced IFNα production by pDC from SLE patients. Resting or the addition of IFNα, IFNγ, and GM-CSF could also recover IFNα-producing ability of pDC from SLE patients to some extent [157, 159]. This suggests that the deficiency of IFNα production from pDC is reversible. Moreover, IFNα production by pDC from SLE versus HC was comparable upon stimulation with influenza viruses or TLR7 agonist [43, 158]. It is possible that pDC in SLE patients and lupus-prone mice can still produce a normal level of IFNα through the TLR7 pathway. Collectively, the results of these studies have raised two important questions: (1) Do pDCs gradually lose the ability to produce IFNα *in vivo* during lupus progression? (2) If pDC fail to produce IFNα in late stage lupus, what is the source of IFNα that stays at a high level in SLE patients and lupus-prone mice? [94].

4.5. Possible IFNα Production from Cells Other Than pDC in Lupus. An early study showed that PBMC from SLE patients could still produce detectable IFNα when pDCs were depleted, suggesting that other cell types besides pDC may have the ability to produce IFNα in SLE [43]. Neutrophils isolated from HC, SLE patients, and B6 mice were able to do so upon nucleosomes or CpG-B stimulation [161]. Interestingly, neutrophils from TLR9-deficient mice retained their ability to produce IFNα upon nucleosomes stimulation, suggesting that the production IFNα in neutrophils is TLR9-independent. Moreover, neutrophils from both SLE patients and lupus-prone mice possessed increased IFNα transcript level compared to HC, although the protein level of IFNα was

not measured in these studies [162–164]. Besides neutrophils, monocytes and cDC can also produce IFNα. With IFNβ priming, monocytes purified from healthy human PBMC, as well as cDC derived *in vitro* from bone marrow of normal mice, were shown to produce IFNα through LPS-activated TLR4 pathway [165]. Monocytes from healthy human PBMC also produced IFNα upon stimulation with liposome-coated RNA [166]. In addition, Ly6C^{high} monocytes are the primary source of IFNα in pristine-induced lupus-prone mice, as depletion of these monocytes abrogated IFNα production [167]. cDC, on the other hand, have been shown to produce IFNα through a cytosolic pattern recognition pathway via stimulator of interferon genes (STING) [168].

4.6. Potential Treatment Strategies of Lupus by Targeting pDC and IFNα. Due to the critical role of pDC and IFNα in the development of lupus, potential treatment strategies targeting them have been proposed. One example is intravenous immunoglobulin (IVIG) therapy, where IgG, the major antibody in IVIG, inhibits IC- or CpG-A-mediated production of IFNα from pDC [129]. It has been suggested that Fc fragment of IgG through blocking FcγRIIA on pDCs directly suppresses the uptake of nucleic acid-containing ICs by pDC [169]. Through the function of IgG glycan hydrolysis, Endoglycosidase S (Endo S) can also inhibit the uptake of ICs [170]. Sialylated subfraction positive (SNA$^+$) Fab$'$ fragment of IgG, targeting unknown receptor on monocytes, induces production of PGE2 by monocytes, which in turn suppresses TLR7/9 agonist-mediated IFNα production by pDC. Another potential treatment targeting pDC and IFNα is DNA-like class R inhibitory oligonucleotides (INH-ODNs), which block TRL7/9-mediated activation of pDC upon stimulation with nucleic acid-containing ICs [171]. Administration of INH-ODN in MRL/lpr lupus-prone mice dramatically ameliorated lupus disease with reduced pathology and autoantibodies. Moreover, proteasome inhibitors have been shown to suppress IFNα production from normal pDC by inhibiting TLR9 translocation from endoplasmic reticulum to endosomes and lysosomes [172, 173]. Furthermore, HMG-CoA reductase inhibitors (statins) and histone deacetylases inhibitors can suppress IFNα production by healthy human pDC through inhibiting IRF7 translocation into the nucleus [174, 175]. Lastly, by neutralizing IFNα directly, sifalimumab, a monoclonal antibody against human IFNα, was able to reduce IFN-signature in phase I clinical trial [176].

A strategy to induce tolerogenic pDC has also been proposed. Subcutaneous injection of H471-94 peptide from histone proteins into NSF1 lupus-prone mice at a low dose induced tolerogenic pDC that promoted Treg cells [177]. Adoptive transfer of tolerogenic pDC into lupus-prone mice was able to reduce autoantibodies against DNA-containing antigens, decrease IL-17 production in spleen, and delay the development of lupus nephritis [177].

5. Open Questions

Many questions remain regarding the mechanisms by which DC modulate lupus pathogenesis that needs to be revealed by additional studies. The first question is whether and how selective depletion of cDC would affect lupus. Many different lupus-prone mouse models have been generated, making it feasible to investigate whether DC are important for lupus development *in vivo*. Depletion studies of whole DC populations, including both cDC and pDC, in MRL/lpr lupus-prone mice suggest the involvement of DC in promoting lupus development, but not activation of naïve T cells. Two additional studies that selectively deplete pDC or abrogate IFNα-producing ability of pDC in lupus-prone mouse models other than MRL/lpr further demonstrate the importance of pDC in lupus pathogenesis. However, selective depletion of cDC populations in lupus-prone mice has not been reported.

The second question is which TLR, TLR9, or TLR7 is critical for the role of pDC in lupus pathogenesis. Studies have shown that the pathogenic role of TLR7 in lupus-prone mice is partially dependent on IFNα induction, and TLR9 on the contrary can regulate lupus progression by suppressing TLR7 signaling [178–181]. However, pDC-specific TLR7 or TLR9 deficiency in lupus-prone mice has not been reported, as B cells and some other innate immune cell types also express TLR7 and TLR9.

A third question is how to develop new treatment strategies targeting DC populations for lupus. Current treatments for lupus are nonspecific immunosuppressive drugs that suppress general immune responses from both innate and adaptive immune system. Side effects, including increases susceptibility to cancers and/or infections, can be severe. Future direction for lupus treatment should be focused on specific targeting with minimal side effects, where DC are a valuable target. New drugs targeting DC should avoid blocking the mechanism by which they defend against pathogens or cancer cells. Therefore, a better understanding of how DC are activated in lupus versus cancer/infection will be particularly useful.

How to translate results obtained from *in vitro* studies is another question. Through either purifying DC directly from PBMC of SLE patients or *in vitro* generating moDC, researchers have investigated activation of DC by self-antigens, activation/maturation markers on DC, cytokine production by DC, and the ability of DC to activate T cells. Similar studies have also been done with bone marrow cells or sorted splenic DC from lupus-prone mice. However, the results from different studies are not always consistent or even contradictory to each other, likely due to differences in stimulation protocols. It is also unclear whether *in vitro* stimulation methods would create the actual environment for DC in SLE patients or lupus-prone mice. In many cases, *in vitro* studies have revealed that the stimuli for DC activation have to be of certain concentrations or given at specific time points, making it difficult to translate the results.

6. Summary

Based on the reviewed studies above, we summarize how cDC and pDC may be involved in lupus pathogenesis. At the initiation stage of lupus, dysregulated cDC and pDC are activated by accumulated self-antigens (e.g., self-nucleic acids bound with associated molecules) and cytokines in genetically predisposed individuals and accumulate in peripheral

immune and nonimmune tissues. Activated pDCs through secreting IFNα then provide immunogenic signals to other immune cells including cDC, monocytes, neutrophils, T cells, and B cells. These leukocytes further promote the activation of pDC and IFNα production. With increasing inflammation, monocytes differentiate into activated cDC, which, together with CDP-derived activated cDC, sustain and amplify primed adaptive immune responses in both immune and nonimmune tissues, thus exacerbating the disease.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] G. C. Tsokos, "Systemic lupus erythematosus," *The New England Journal of Medicine*, vol. 365, no. 22, pp. 2110–2121, 2011.

[2] G. K. W. Lam and M. Petri, "Assessment of systemic lupus erythematosus," *Clinical and Experimental Rheumatology*, vol. 23, no. 5, supplement 39, pp. S120–S132, 2005.

[3] M. J. Shlomchik, J. E. Craft, and M. J. Mamula, "From T to B and back again: positive feedback in systemic autoimmune disease," *Nature Reviews Immunology*, vol. 1, no. 2, pp. 147–153, 2001.

[4] Z. Liu and A. Davidson, "Taming lupus-a new understanding of pathogenesis is leading to clinical advances," *Nature Medicine*, vol. 18, no. 6, pp. 871–882, 2012.

[5] A. Rahman and D. A. Isenberg, "Systemic lupus erythematosus," *The New England Journal of Medicine*, vol. 358, no. 9, pp. 929–939, 2008.

[6] T. K. Nowling and G. S. Gilkeson, "Mechanisms of tissue injury in lupus nephritis," *Arthritis Research & Therapy*, vol. 13, no. 6, article 250, 2011.

[7] K. Inaba and R. M. Steinman, "Protein-specific helper T-lymphocyte formation initiated by dendritic cells," *Science*, vol. 229, no. 4712, pp. 475–479, 1985.

[8] M. Merad, P. Sathe, J. Helft, J. Miller, and A. Mortha, "The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting," *Annual Review of Immunology*, vol. 31, pp. 563–604, 2013.

[9] A. N. Theofilopoulos, R. Baccala, B. Beutler, and D. H. Kono, "Type I interferons (α/β) in immunity and autoimmunity," *Annual Review of Immunology*, vol. 23, pp. 307–336, 2005.

[10] G. Grouard, M.-C. Rissoan, L. Filgueira, I. Durand, J. Banchereau, and Y.-J. Liu, "The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand," *Journal of Experimental Medicine*, vol. 185, no. 6, pp. 1101–1111, 1997.

[11] D. Ganguly, S. Haak, V. Sisirak, and B. Reizis, "The role of dendritic cells in autoimmunity," *Nature Reviews Immunology*, vol. 13, no. 8, pp. 566–577, 2013.

[12] T. Birnberg, L. Bar-On, A. Sapoznikov et al., "Lack of conventional dendritic cells is compatible with normal development and T cell homeostasis, but causes myeloid proliferative syndrome," *Immunity*, vol. 29, no. 6, pp. 986–997, 2008.

[13] C. Ohnmacht, A. Pullner, S. B. S. King et al., "Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity," *The Journal of Experimental Medicine*, vol. 206, no. 3, pp. 549–559, 2009.

[14] L. L. Teichmann, M. L. Ols, M. Kashgarian, B. Reizis, D. H. Kaplan, and M. J. Shlomchik, "Dendritic cells in lupus are not required for activation of T and B cells but promote their expansion, resulting in tissue damage," *Immunity*, vol. 33, no. 6, pp. 967–978, 2010.

[15] J. Yan, B. P. Harvey, R. J. Gee, M. J. Shlomchik, and M. J. Mamula, "B cells drive early T cell autoimmunity in vivo prior to dendritic cell-mediated autoantigen presentation," *The Journal of Immunology*, vol. 177, no. 7, pp. 4481–4487, 2006.

[16] S. L. Rowland, J. M. Riggs, S. Gilfillan et al., "Early, transient depletion of plasmacytoid dendritic cells ameliorates autoimmunity in a lupus model," *The Journal of Experimental Medicine*, vol. 211, no. 10, pp. 1977–1991, 2014.

[17] L. M. Davison and T. N. Jørgensen, "Sialic acid-binding immunoglobulin-type lectin H-positive plasmacytoid dendritic cells drive spontaneous lupus-like disease development in B6.Nba2 mice," *Arthritis & Rheumatology*, vol. 67, no. 4, pp. 1012–1022, 2015.

[18] J. C. Crispín, M. I. Vargas-Rojas, A. Monsiváis-Urenda, and J. Alcocer-Varela, "Phenotype and function of dendritic cells of patients with systemic lupus erythematosus," *Clinical Immunology*, vol. 143, no. 1, pp. 45–50, 2012.

[19] K. Migita, T. Miyashita, Y. Maeda et al., "Reduced blood BDCA-2^+ (lymphoid) and CD11c$^+$ (myeloid) dendritic cells in systemic lupus erythematosus," *Clinical and Experimental Immunology*, vol. 142, no. 1, pp. 84–91, 2005.

[20] N. Fiore, G. Castellano, A. Blasi et al., "Immature myeloid and plasmacytoid dendritic cells infiltrate renal tubulointerstitium in patients with lupus nephritis," *Molecular Immunology*, vol. 45, no. 1, pp. 259–265, 2008.

[21] O. Jin, S. Kavikondala, L. Sun et al., "Systemic lupus erythematosus patients have increased number of circulating plasmacytoid dendritic cells, but decreased myeloid dendritic cells with deficient CD83 expression," *Lupus*, vol. 17, no. 7, pp. 654–662, 2008.

[22] S. A. Khan, J. Nowatzky, S. Jiménez-Branda et al., "Active systemic lupus erythematosus is associated with decreased blood conventional dendritic cells," *Experimental and Molecular Pathology*, vol. 95, no. 2, pp. 121–123, 2013.

[23] A. Henriques, L. Inês, T. Carvalheiro et al., "Functional characterization of peripheral blood dendritic cells and monocytes in systemic lupus erythematosus," *Rheumatology International*, vol. 32, no. 4, pp. 863–869, 2012.

[24] S. Segerer, F. Heller, M. T. Lindenmeyer et al., "Compartment specific expression of dendritic cell markers in human glomerulonephritis," *Kidney International*, vol. 74, no. 1, pp. 37–46, 2008.

[25] L. Schiffer, R. Bethunaickan, M. Ramanujam et al., "Activated renal macrophages are markers of disease onset and disease remission in lupus nephritis," *The Journal of Immunology*, vol. 180, no. 3, pp. 1938–1947, 2008.

[26] R. Bethunaickan, C. C. Berthier, M. Ramanujam et al., "A unique hybrid renal mononuclear phagocyte activation phenotype in murine systemic lupus erythematosus nephritis," *The Journal of Immunology*, vol. 186, no. 8, pp. 4994–5003, 2011.

[27] R. Sahu, R. Bethunaickan, S. Singh, and A. Davidson, "Structure and function of renal macrophages and dendritic cells from lupus-prone mice," *Arthritis and Rheumatology*, vol. 66, no. 6, pp. 1596–1607, 2014.

[28] X. Liao, J. Ren, C. H. Wei et al., "Paradoxical effects of all-trans-retinoic acid on lupus-like disease in the MRL/lpr mouse model," *PLoS ONE*, vol. 10, no. 3, Article ID e0118176, 2015.

[29] J. A. Deane, P. Pisitkun, R. S. Barrett et al., "Control of toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation," *Immunity*, vol. 27, no. 5, pp. 801–810, 2007.

[30] M. B. Buechler, T. H. Teal, K. B. Elkon, and J. A. Hamerman, "Cutting edge: type i ifn drives emergency myelopoiesis and peripheral myeloid expansion during chronic tlr7 signaling," *The Journal of Immunology*, vol. 190, no. 3, pp. 886–891, 2013.

[31] S. L. Kalled, A. H. Cutler, and L. C. Burkly, "Apoptosis and altered dendritic cell homeostasis in lupus nephritis are limited by anti-CD154 treatment," *Journal of Immunology*, vol. 167, no. 3, pp. 1740–1747, 2001.

[32] M. R. Clatworthy, C. E. P. Aronin, R. J. Mathews, N. Y. Morgan, K. G. C. Smith, and R. N. Germain, "Immune complexes stimulate CCR7-dependent dendritic cell migration to lymph nodes," *Nature Medicine*, vol. 20, no. 12, pp. 1458–1463, 2014.

[33] S. Gonzalvo-Feo, A. Del Prete, M. Pruenster et al., "Endothelial cell-derived chemerin promotes dendritic cell transmigration," *Journal of Immunology*, vol. 192, no. 5, pp. 2366–2373, 2014.

[34] G. De Palma, G. Castellano, A. Del Prete et al., "The possible role of ChemR23/Chemerin axis in the recruitment of dendritic cells in lupus nephritis," *Kidney International*, vol. 79, no. 11, pp. 1228–1235, 2011.

[35] A. Rodriguez-Pla, P. Patel, H. T. Maecker et al., "IFN priming is necessary but not sufficient to turn on a migratory dendritic cell program in lupus monocytes," *Journal of Immunology*, vol. 192, no. 12, pp. 5586–5598, 2014.

[36] D. Ding, H. Mehta, W. J. McCune, and M. J. Kaplan, "Aberrant phenotype and function of myeloid dendritic cells in systemic lupus erythematosus," *The Journal of Immunology*, vol. 177, no. 9, pp. 5878–5889, 2006.

[37] L. Colonna, J.-A. Dinnal, D. K. Shivers, L. Frisoni, R. Caricchio, and S. Gallucci, "Abnormal costimulatory phenotype and function of dendritic cells before and after the onset of severe murine lupus," *Arthritis Research and Therapy*, vol. 8, no. 2, article R49, 2006.

[38] M. A. Gleisner, P. Reyes, J. Alfaro et al., "Dendritic and stromal cells from the spleen of lupic mice present phenotypic and functional abnormalities," *Molecular Immunology*, vol. 54, no. 3-4, pp. 423–434, 2013.

[39] S. Wan, C. Xia, and L. Morel, "IL-6 produced by dendritic cells from lupus-prone mice inhibits $CD4^+CD25^+$ T cell regulatory functions," *Journal of Immunology*, vol. 178, no. 1, pp. 271–279, 2007.

[40] Y. Berkun, I. Verbovetski, A. Ben-Ami et al., "Altered dendritic cells with tolerizing phenotype in patients with systemic lupus erythematosus," *European Journal of Immunology*, vol. 38, no. 10, pp. 2896–2904, 2008.

[41] L. J. Carreno, R. Pacheco, M. A. Gutierrez, S. Jacobelli, and A. M. Kalergis, "Disease activity in systemic lupus erythematosus is associated with an altered expression of low-affinity Fcγ receptors and costimulatory molecules on dendritic cells," *Immunology*, vol. 128, no. 3, pp. 334–341, 2009.

[42] F. Sallusto and A. Lanzavecchia, "Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α," *The Journal of Experimental Medicine*, vol. 179, no. 4, pp. 1109–1118, 1994.

[43] P. Blanco, A. K. Palucka, M. Gill, V. Pascual, and J. Banchereau, "Induction of dendritic cell differentiation by IFN-α in systemic lupus erythematosus," *Science*, vol. 294, no. 5546, pp. 1540–1543, 2001.

[44] G.-M. Deng, L. Liu, V. C. Kyttaris, and G. C. Tsokos, "Lupus serum IgG induces skin inflammation through the TNFR1 signaling pathway," *The Journal of Immunology*, vol. 184, no. 12, pp. 7154–7161, 2010.

[45] H. Joo, C. Coquery, Y. Xue et al., "Serum from patients with SLE instructs monocytes to promote IgG and IgA plasmablast differentiation," *The Journal of Experimental Medicine*, vol. 209, no. 7, pp. 1335–1348, 2012.

[46] V. Urbonaviciute, B. G. Fürnrohr, S. Meister et al., "Induction of inflammatory and immune responses by HMGB1-nucleosome complexes: implications for the pathogenesis of SLE," *The Journal of Experimental Medicine*, vol. 205, no. 13, pp. 3007–3018, 2008.

[47] J. H. Fransen, L. B. Hilbrands, J. Ruben et al., "Mouse dendritic cells matured by ingestion of apoptotic blebs induce T cells to produce interleukin-17," *Arthritis & Rheumatism*, vol. 60, no. 8, pp. 2304–2313, 2009.

[48] J. H. Fransen, L. B. Hilbrands, C. W. Jacobs, G. J. Adema, J. H. Berden, and J. Van der Vlag, "Both early and late apoptotic blebs are taken up by DC and induce IL-6 production," *Autoimmunity*, vol. 42, no. 4, pp. 325–327, 2009.

[49] S. Ivanov, A.-M. Dragoi, X. Wang et al., "A novel role for HMGB1 in TLR9-mediated inflammatory responses to CpG-DNA," *Blood*, vol. 110, no. 6, pp. 1970–1981, 2007.

[50] K. Yasuda, C. Richez, M. B. Uccellini et al., "Requirement for DNA CpG content in TLR9-dependent dendritic cell activation induced by DNA-containing immune complexes," *Journal of Immunology*, vol. 183, no. 5, pp. 3109–3117, 2009.

[51] P. Decker, H. Singh-Jasuja, S. Haager, I. Kötter, and H.-G. Rammensee, "Nucleosome, the main autoantigen in systemic lupus erythematosus, induces direct dendritic cell activation via a MyD88-independent pathway: consequences on inflammation," *The Journal of Immunology*, vol. 174, no. 6, pp. 3326–3334, 2005.

[52] K. Kis-Toth, A. Szanto, T.-H. Thai, and G. C. Tsokos, "Cytosolic DNA-activated human dendritic cells are potent activators of the adaptive immune response," *The Journal of Immunology*, vol. 187, no. 3, pp. 1222–1234, 2011.

[53] E. Savarese, O.-W. Chae, S. Trowitzsch et al., "U1 small nuclear ribonucleoprotein immune complexes induce type I interferon in plasmacytoid dendritic cells through TLR7," *Blood*, vol. 107, no. 8, pp. 3229–3234, 2006.

[54] D. Ganguly, G. Chamilos, R. Lande et al., "Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8," *The Journal of Experimental Medicine*, vol. 206, no. 9, pp. 1983–1994, 2009.

[55] J. Vas, C. Grönwall, A. Marshak-Rothstein, and G. J. Silverman, "Natural antibody to apoptotic cell membranes inhibits the proinflammatory properties of lupus autoantibody immune complexes," *Arthritis and Rheumatism*, vol. 64, no. 10, pp. 3388–3398, 2012.

[56] C. Gronwall, Y. Chen, J. Vas et al., "MAPK phosphatase-1 is required for regulatory natural autoantibody-mediated inhibition of TLR responses," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 48, pp. 19745–19750, 2012.

[57] E. V. Clarke, B. M. Weist, C. M. Walsh, and A. J. Tenner, "Complement protein C1q bound to apoptotic cells suppresses human macrophage and dendritic cell-mediated Th17 and Th1

T cell subset proliferation," *Journal of Leukocyte Biology*, vol. 97, no. 1, pp. 147–160, 2015.

[58] B. Desnues, A. B. Macedo, A. Roussel-Queval et al., "TLR8 on dendritic cells and TLR9 on B cells restrain TLR7-mediated spontaneous autoimmunity in C57BL/6 mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 4, pp. 1497–1502, 2014.

[59] L. L. Teichmann, D. Schenten, R. Medzhitov, M. Kashgarian, and M. J. Shlomchik, "Signals via the adaptor MyD88 in B cells and DCs make distinct and synergistic contributions to immune activation and tissue damage in lupus," *Immunity*, vol. 38, no. 3, pp. 528–540, 2013.

[60] C. Lamagna, P. Scapini, J. A. van Ziffle, A. L. DeFranco, and C. A. Lowell, "Hyperactivated MyD88 signaling in dendritic cells, through specific deletion of Lyn kinase, causes severe autoimmunity and inflammation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 35, pp. E3311–E3320, 2013.

[61] Y. Tada, S. Kondo, S. Aoki et al., "Interferon regulatory factor 5 is critical for the development of lupus in MRL/lpr mice," *Arthritis and Rheumatism*, vol. 63, no. 3, pp. 738–748, 2011.

[62] M. Kool, G. van Loo, W. Waelput et al., "The ubiquitin-editing protein A20 prevents dendritic cell activation, recognition of apoptotic cells, and systemic autoimmunity," *Immunity*, vol. 35, no. 1, pp. 82–96, 2011.

[63] S. J. Kim, P. K. Gregersen, and B. Diamond, "Regulation of dendritic cell activation by microRNA let-7c and BLIMP1," *The Journal of Clinical Investigation*, vol. 123, no. 2, pp. 823–833, 2013.

[64] S. J. Kim, Y. R. Zou, J. Goldstein, B. Reizis, and B. Diamond, "Tolerogenic function of Blimp-1 in dendritic cells," *The Journal of Experimental Medicine*, vol. 208, no. 11, pp. 2193–2199, 2011.

[65] M. Chen, L. Huang, and J. Wang, "Deficiency of Bim in dendritic cells contributes to overactivation of lymphocytes and autoimmunity," *Blood*, vol. 109, no. 10, pp. 4360–4367, 2007.

[66] B. P. dos Santos, J. V. Valverde, P. Rohr et al., "TLR7/8/9 polymorphisms and their associations in systemic lupus erythematosus patients from Southern Brazil," *Lupus*, vol. 21, no. 3, pp. 302–309, 2012.

[67] A. Kawasaki, C. Kyogoku, J. Ohashi et al., "Association of IRF5 polymorphisms with systemic lupus erythematosus in a Japanese population: support for a crucial role of intron 1 polymorphisms," *Arthritis & Rheumatism*, vol. 58, no. 3, pp. 826–834, 2008.

[68] S. L. Musone, K. E. Taylor, T. T. Lu et al., "Multiple polymorphisms in the TNFAIP3 region are independently associated with systemic lupus erythematosus," *Nature Genetics*, vol. 40, no. 9, pp. 1062–1064, 2008.

[69] R. Lu, G. S. Vidal, J. A. Kelly et al., "Genetic associations of LYN with systemic lupus erythematosus," *Genes and Immunity*, vol. 10, no. 5, pp. 397–403, 2009.

[70] V. Gateva, J. K. Sandling, G. Hom et al., "A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus," *Nature Genetics*, vol. 41, no. 11, pp. 1228–1233, 2009.

[71] M. A. Jensen, K. C. Patterson, A. A. Kumar, M. Kumabe, B. S. Franek, and T. B. Niewold, "Functional genetic polymorphisms in ILT3 are associated with decreased surface expression on dendritic cells and increased serum cytokines in lupus patients," *Annals of the Rheumatic Diseases*, vol. 72, no. 4, pp. 596–601, 2013.

[72] H. Xie, C. Hua, L. Sun et al., "17β-estradiol induces CD40 expression in dendritic cells via MAPK signaling pathways in a minichromosome maintenance protein 6-dependent manner," *Arthritis & Rheumatism*, vol. 63, no. 8, pp. 2425–2435, 2011.

[73] E. Csomor, Z. Bajtay, N. Sándor et al., "Complement protein C1q induces maturation of human dendritic cells," *Molecular Immunology*, vol. 44, no. 13, pp. 3389–3397, 2007.

[74] B. K. Teh, J. G. Yeo, L. M. Chern, and J. Lu, "C1q regulation of dendritic cell development from monocytes with distinct cytokine production and T cell stimulation," *Molecular Immunology*, vol. 48, no. 9-10, pp. 1128–1138, 2011.

[75] P. B. Stranges, J. Watson, C. J. Cooper et al., "Elimination of antigen-presenting cells and autoreactive T cells by Fas contributes to prevention of autoimmunity," *Immunity*, vol. 26, no. 5, pp. 629–641, 2007.

[76] M. Chen, K. Felix, and J. Wang, "Immune regulation through mitochondrion-dependent dendritic cell death induced by T regulatory cells," *The Journal of Immunology*, vol. 187, no. 11, pp. 5684–5692, 2011.

[77] M. Chen, Y.-H. Wang, Y. Wang et al., "Dendritic cell apoptosis in the maintenance of immune tolerance," *Science*, vol. 311, no. 5764, pp. 1160–1164, 2006.

[78] P. Yang, B. Li, P. Lv, Y. Zhang, and X.-M. Gao, "Interaction between antigen presenting cells and autoreactive T cells derived from BXSB mice with murine lupus," *Cell Research*, vol. 17, no. 6, pp. 556–564, 2007.

[79] T.-C. Tzeng, J.-L. Suen, and B.-L. Chiang, "Dendritic cells pulsed with apoptotic cells activate self-reactive T-cells of lupus mice both in vitro and in vivo," *Rheumatology*, vol. 45, no. 10, pp. 1230–1237, 2006.

[80] L. Estrada-Capetillo, B. Hernández-Castro, A. Monsiváis-Urenda et al., "Induction of Th17 lymphocytes and treg cells by monocyte-derived dendritic cells in patients with rheumatoid arthritis and systemic lupus erythematosus," *Clinical and Developmental Immunology*, vol. 2013, Article ID 584303, 9 pages, 2013.

[81] S. Wan, Z. Zhou, B. Duan, and L. Morel, "Direct B cell stimulation by dendritic cells in a mouse model of lupus," *Arthritis and Rheumatism*, vol. 58, no. 6, pp. 1741–1750, 2008.

[82] A. Sang, Y.-Y. Zheng, Y. Yin et al., "Dysregulated cytokine production by dendritic cells modulates B cell responses in the NZM2410 mouse model of lupus," *PLoS ONE*, vol. 9, no. 8, Article ID e102151, 2014.

[83] M. M. Gilbert, D. G. Carnathan, P. C. Cogswell, L. Lin, A. S. Baldwin Jr., and B. J. Vilen, "Dendritic cells from lupus-prone mice are defective in repressing immunoglobulin secretion," *The Journal of Immunology*, vol. 178, no. 8, pp. 4803–4810, 2007.

[84] Y. Iwata, K. Furuichi, N. Sakai et al., "Dendritic cells contribute to autoimmune kidney injury in MRL-Fas^{lpr} mice," *The Journal of Rheumatology*, vol. 36, no. 2, pp. 306–314, 2009.

[85] M. Look, E. Stern, Q. A. Wang et al., "Nanogel-based delivery of mycophenolic acid ameliorates systemic lupus erythematosus in mice," *The Journal of Clinical Investigation*, vol. 123, no. 4, pp. 1741–1749, 2013.

[86] M. Look, W. M. Saltzman, J. Craft, and T. M. Fahmy, "The nanomaterial-dependent modulation of dendritic cells and its potential influence on therapeutic immunosuppression in lupus," *Biomaterials*, vol. 35, no. 3, pp. 1089–1095, 2014.

[87] Y. Zhang, S. Liu, Y. Yu et al., "Immune complex enhances tolerogenecity of immature dendritic cells via FcγRIIb and promotes FcγRIIb-overexpressing dendritic cells to attenuate lupus," *European Journal of Immunology*, vol. 41, no. 4, pp. 1154–1164, 2011.

[88] Y. Xia, S. Jiang, S. Weng, X. Lv, H. Cheng, and C. Fang, "Antigen-specific immature dendritic cell vaccine ameliorates anti-dsDNA antibody-induced renal damage in a mouse model," *Rheumatology*, vol. 50, no. 12, pp. 2187–2196, 2011.

[89] U. Sela, A. Sharabi, M. Dayan, R. Hershkoviz, and E. Mozes, "The role of dendritic cells in the mechanism of action of a peptide that ameliorates lupus in murine models," *Immunology*, vol. 128, no. 1, supplement, pp. e395–e405, 2009.

[90] H. J. Wu, Y. Lo, D. Luk, C. S. Lau, L. Lu, and M. Y. Mok, "Alternatively activated dendritic cells derived from systemic lupus erythematosus patients have tolerogenic phenotype and function," *Clinical Immunology*, vol. 156, no. 1, pp. 43–57, 2015.

[91] K. Santana-de Anda, D. Gómez-Martín, A. E. Monsivais-Urenda, M. Salgado-Bustamante, R. González-Amaro, and J. Alcocer-Varela, "Interferon regulatory factor 3 as key element of the interferon signature in plasmacytoid dendritic cells from systemic lupus erythematosus patients: novel genetic associations in the Mexican mestizo population," *Clinical and Experimental Immunology*, vol. 178, no. 3, pp. 428–437, 2014.

[92] T. Carvalheiro, A. Rodrigues, A. Lopes et al., "Tolerogenic versus inflammatory activity of peripheral blood monocytes and dendritic cells subpopulations in systemic lupus erythematosus," *Clinical and Developmental Immunology*, vol. 2012, Article ID 934161, 14 pages, 2012.

[93] S. Blomberg, M.-L. Eloranta, M. Magnusson, G. V. Alm, and L. Ronnblom, "Expression of the markers BDCA-2 and BDCA-4 and production of interferon-α by plasmacytoid dendritic cells in systemic lupus erythematosus," *Arthritis & Rheumatism*, vol. 48, no. 9, pp. 2524–2532, 2003.

[94] P. Wu, J. Wu, S. Liu et al., "TLR9/TLR7-triggered downregulation of BDCA2 expression on human plasmacytoid dendritic cells from healthy individuals and lupus patients," *Clinical Immunology*, vol. 129, no. 1, pp. 40–48, 2008.

[95] M. Tucci, C. Quatraro, L. Lombardi, C. Pellegrino, F. Dammacco, and F. Silvestris, "Glomerular accumulation of plasmacytoid dendritic cells in active lupus nephritis: role of interleukin-18," *Arthritis and Rheumatism*, vol. 58, no. 1, pp. 251–262, 2008.

[96] W. Vermi, S. Lonardi, M. Morassi et al., "Cutaneous distribution of plasmacytoid dendritic cells in lupus erythematosus. Selective tropism at the site of epithelial apoptotic damage," *Immunobiology*, vol. 214, no. 9-10, pp. 877–886, 2009.

[97] Q. Yin, X. Xu, Y. Lin, J. Lv, L. Zhao, and R. He, "Ultraviolet B irradiation induces skin accumulation of plasmacytoid dendritic cells: a possible role for chemerin," *Autoimmunity*, vol. 47, no. 3, pp. 185–192, 2014.

[98] J. W. Park, S. Y. Moon, J. H. Lee et al., "Bone marrow analysis of immune cells and apoptosis in patients with systemic lupus erythematosus," *Lupus*, vol. 23, no. 10, pp. 975–985, 2014.

[99] T. Ito, H. Kanzler, O. Duramad, W. Cao, and Y.-J. Liu, "Specialization, kinetics, and repertoire of type 1 interferon responses by human plasmacytoid predendritic cells," *Blood*, vol. 107, no. 6, pp. 2423–2431, 2006.

[100] H. Agrawal, N. Jacob, E. Carreras et al., "Deficiency of type I IFN receptor in lupus-prone New Zealand mixed 2328 mice decreases dendritic cell numbers and activation and protects from disease," *The Journal of Immunology*, vol. 183, no. 9, pp. 6021–6029, 2009.

[101] E. Pau, Y.-H. Cheung, C. Loh, G. Lajoie, and J. E. Wither, "TLR tolerance reduces IFN-alpha production despite plasmacytoid dendritic cell expansion and anti-nuclear antibodies in NZB bicongenic mice," *PLoS ONE*, vol. 7, no. 5, Article ID e36761, 2012.

[102] Y. Zhan, E. M. Carrington, H. Ko et al., "Bcl-2 antagonists kill plasmacytoid dendritic cells from lupus-prone mice and dampen interferon-alpha production," *Arthritis & Rheumatology*, vol. 67, no. 3, pp. 797–808, 2015.

[103] C. Guiducci, M. Gong, Z. Xu et al., "TLR recognition of self nucleic acids hampers glucocorticoid activity in lupus," *Nature*, vol. 465, no. 7300, pp. 937–941, 2010.

[104] Y. Hong, J. Wu, J. Zhao et al., "miR-29b and miR-29c are involved in Toll-like receptor control of glucocorticoid-induced apoptosis in human plasmacytoid dendritic cells," *PLoS ONE*, vol. 8, no. 7, Article ID e69926, 2013.

[105] Y. Lepelletier, R. Zollinger, C. Ghirelli et al., "Toll-like receptor control of glucocorticoid-induced apoptosis in human plasmacytoid predendritic cells (pDCs)," *Blood*, vol. 116, no. 18, pp. 3389–3397, 2010.

[106] 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.

[107] F. Kanakoudi-Tsakalidou, E. Farmaki, V. Tzimouli et al., "Simultaneous changes in serum HMGB1 and IFN-α levels and in LAIR-1 expression on plasmatoid dendritic cells of patients with juvenile SLE.. New therapeutic options?" *Lupus*, vol. 23, no. 3, pp. 305–312, 2014.

[108] G. S. Garcia-Romo, S. Caielli, B. Vega et al., "Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus," *Science Translational Medicine*, vol. 3, no. 73, Article ID 73ra20, 2011.

[109] X. Liao, S. Li, R. E. Settlage et al., "Cutting edge: plasmacytoid dendritic cells in late-stage lupus mice defective in producing IFN-α," *The Journal of Immunology*, vol. 195, no. 10, pp. 4578–4582, 2015.

[110] L. E. Wilson, D. Widman, S. H. Dikman, and P. D. Gorevic, "Autoimmune disease complicating antiviral therapy for hepatitis C virus infection," *Seminars in Arthritis and Rheumatism*, vol. 32, no. 3, pp. 163–173, 2002.

[111] L. E. Rönnblom, G. V. Alm, and K. E. Öberg, "Possible induction of systemic lupus erythematosus by interferon-α treatment in a patient with a malignant carcinoid tumour," *Journal of Internal Medicine*, vol. 227, no. 3, pp. 207–210, 1990.

[112] A. Mathian, A. Weinberg, M. Gallegos, J. Banchereau, and S. Koutouzov, "IFN-α induces early lethal lupus in preautoimmune (New Zealand Black x New Zealand White) F1 but not in BALB/c mice," *Journal of Immunology*, vol. 174, no. 5, pp. 2499–2506, 2005.

[113] M.-L. Santiago-Raber, R. Baccala, K. M. Haraldsson et al., "Type-I interferon receptor deficiency reduces lupus-like disease in NZB mice," *The Journal of Experimental Medicine*, vol. 197, no. 6, pp. 777–788, 2003.

[114] D. Braun, P. Geraldes, and J. Demengeot, "Type I Interferon controls the onset and severity of autoimmune manifestations in lpr mice," *Journal of Autoimmunity*, vol. 20, no. 1, pp. 15–25, 2003.

[115] J. D. Hron and S. L. Peng, "Type I IFN protects against murine lupus," *The Journal of Immunology*, vol. 173, no. 3, pp. 2134–2142, 2004.

[116] R. Baccala, R. Gonzalez-Quintial, R. D. Schreiber, B. R. Lawson, D. H. Kono, and A. N. Theofilopoulos, "Anti-IFN-α/β receptor

antibody treatment ameliorates disease in lupus-predisposed mice," *Journal of Immunology*, vol. 189, no. 12, pp. 5976–5984, 2012.

[117] V. Sisirak, D. Ganguly, K. L. Lewis et al., "Genetic evidence for the role of plasmacytoid dendritic cells in systemic lupus erythematosus," *The Journal of Experimental Medicine*, vol. 211, no. 10, pp. 1969–1976, 2014.

[118] D. M. Santer, A. E. Wiedeman, T. H. Teal, P. Ghosh, and K. B. Elkon, "Plasmacytoid dendritic cells and C1q differentially regulate inflammatory gene induction by lupus immune complexes," *Journal of Immunology*, vol. 188, no. 2, pp. 902–915, 2012.

[119] E.-M. Fehr, S. Spoerl, P. Heyder et al., "Apoptotic-cell-derived membrane vesicles induce an alternative maturation of human dendritic cells which is disturbed in SLE," *Journal of Autoimmunity*, vol. 40, no. 1, pp. 86–95, 2013.

[120] L. Abeler-Dörner, C. C. Rieger, B. Berger et al., "Interferon-α abrogates the suppressive effect of apoptotic cells on dendritic cells in an in vitro model of systemic lupus erythematosus pathogenesis," *The Journal of Rheumatology*, vol. 40, no. 10, pp. 1683–1696, 2013.

[121] M. Yamauchi, M. Hashimoto, K. Ichiyama et al., "Ifi202, an IFN-inducible candidate gene for lupus susceptibility in NZB/ W F1 mice, is a positive regulator for NF-κB activation in dendritic cells," *International Immunology*, vol. 19, no. 8, pp. 935–942, 2007.

[122] X. Huang, N. Shen, C. Bao, Y. Gu, L. Wu, and S. Chen, "Interferon-induced protein IFIT4 is associated with systemic lupus erythematosus and promotes differentiation of monocytes into dendritic cell-like cells," *Arthritis Research & Therapy*, vol. 10, no. 4, article R91, 2008.

[123] Y. Hu and L. B. Ivashkiv, "Costimulation of chemokine receptor signaling by matrix metalloproteinase-9 mediates enhanced migration of IFN-α dendritic cells," *The Journal of Immunology*, vol. 176, no. 10, pp. 6022–6033, 2006.

[124] A. J. Mangini, R. Lafyatis, and J. M. Van Seventer, "Type I interferons inhibition of inflammatory T helper cell responses in systemic lupus erythematosus," *Annals of the New York Academy of Sciences*, vol. 1108, pp. 11–23, 2007.

[125] M. Clement, N. Charles, B. Escoubet et al., "CD4+CXCR3+ T cells and plasmacytoid dendritic cells drive accelerated atherosclerosis associated with systemic lupus erythematosus," *Journal of Autoimmunity*, vol. 63, pp. 59–67, 2015.

[126] J. H. Wang, Q. Wu, P. Yang et al., "Type I interferon-dependent CD86^{high} marginal zone precursor B cells are potent T cell costimulators in mice," *Arthritis & Rheumatism*, vol. 63, no. 4, pp. 1054–1064, 2011.

[127] H. Li, Y. X. Fu, Q. Wu et al., "Interferon-induced mechanosensing defects impede apoptotic cell clearance in lupus," *The Journal of Clinical Investigation*, vol. 125, no. 7, pp. 2877–2890, 2015.

[128] T. E. Quan, R. M. Roman, B. J. Rudenga, V. M. Holers, and J. E. Craft, "Epstein-barr virus promotes interferon-α production by plasmacytoid dendritic cells," *Arthritis and Rheumatism*, vol. 62, no. 6, pp. 1693–1701, 2010.

[129] U. Båve, M. Magnusson, M.-L. Eloranta, A. Perers, G. V. Alm, and L. Rönnblom, "FcγRIIa is expressed on natural IFN-α-producing cells (plasmacytoid dendritic cells) and is required for the IFN-α production induced by apoptotic cells combined with lupus IgG," *The Journal of Immunology*, vol. 171, no. 6, pp. 3296–3302, 2003.

[130] T. K. Means, E. Latz, F. Hayashi, M. R. Murali, D. T. Golenbock, and A. D. Luster, "Human lupus autoantibody-DNA complexes activate DCs through cooperation of CD32 and TLR9," *The Journal of Clinical Investigation*, vol. 115, no. 2, pp. 407–417, 2005.

[131] J. Vollmer, S. Tluk, C. Schmitz et al., "Immune stimulation mediated by autoantigen binding sites within small nuclear RNAs involves Toll-like receptors 7 and 8," *The Journal of Experimental Medicine*, vol. 202, no. 11, pp. 1575–1585, 2005.

[132] C. Mold and T. W. D. Clos, "C-reactive protein inhibits plasmacytoid dendritic cell interferon responses to autoantibody immune complexes," *Arthritis and Rheumatism*, vol. 65, no. 7, pp. 1891–1901, 2013.

[133] H. Wang, T. Li, S. Chen, Y. Gu, and S. Ye, "Neutrophil extracellular trap mitochondrial DNA and its autoantibody in systemic lupus erythematosus and a proof-of-concept trial of metformin," *Arthritis & Rheumatology*, vol. 67, no. 12, pp. 3190–3200, 2015.

[134] R. Lande, J. Gregorio, V. Facchinetti et al., "Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide," *Nature*, vol. 449, no. 7162, pp. 564–569, 2007.

[135] R. Lande, D. Ganguly, V. Facchinetti et al., "Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus," *Science Translational Medicine*, vol. 3, no. 73, Article ID 73ra19, 2011.

[136] R. Baccala, R. Gonzalez-Quintial, A. L. Blasius et al., "Essential requirement for IRF8 and SLC15A4 implicates plasmacytoid dendritic cells in the pathogenesis of lupus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 8, pp. 2940–2945, 2013.

[137] A. Sadanaga, H. Nakashima, M. Akahoshi et al., "Protection against autoimmune nephritis in MyD88-deficient MRL/lpr mice," *Arthritis and Rheumatism*, vol. 56, no. 5, pp. 1618–1628, 2007.

[138] Y. Xu, P. Y. Lee, Y. Li et al., "Pleiotropic IFN-dependent and -independent effects of IRF5 on the pathogenesis of experimental lupus," *Journal of Immunology*, vol. 188, no. 8, pp. 4113–4121, 2012.

[139] K. Yasuda, C. Richez, J. W. Maciaszek et al., "Murine dendritic cell type I IFN production induced by human IgG-RNA immune complexes Is IFN regulatory factor (IRF)5 and IRF7 dependent and is required for IL-6 production," *The Journal of Immunology*, vol. 178, no. 11, pp. 6876–6885, 2007.

[140] E. Y. Chiang, X. Yu, and J. L. Grogan, "Immune complex-mediated cell activation from systemic lupus erythematosus and rheumatoid arthritis patients elaborate different requirements for IRAK1/4 kinase activity across human cell types," *The Journal of Immunology*, vol. 186, no. 2, pp. 1279–1288, 2011.

[141] H. Yanai, T. Ban, Z. Wang et al., "HMGB proteins function as universal sentinels for nucleic-acid-mediated innate immune responses," *Nature*, vol. 462, no. 7269, pp. 99–103, 2009.

[142] J. Tian, A. M. Avalos, S.-Y. Mao et al., "Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE," *Nature Immunology*, vol. 8, no. 5, pp. 487–496, 2007.

[143] J. Di Domizio, S. Dorta-Estremera, M. Gagea et al., "Nucleic acid-containing amyloid fibrils potently induce type I interferon and stimulate systemic autoimmunity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 36, pp. 14550–14555, 2012.

[144] C. Orellana, A. Collado, M. V. Hernandez, J. Font, J. A. Del Olmo, and J. Munoz-Gomez, "When does amyloidosis complicate systemic lupus erythematosus?" *Lupus*, vol. 4, no. 5, pp. 415–417, 1995.

[145] S. Gaitonde, D. Samols, and I. Kushner, "C-reactive protein and systemic lupus erythematosus," *Arthritis Care and Research*, vol. 59, no. 12, pp. 1814–1820, 2008.

[146] M. Kirschfink, F. Petry, K. Khirwadkar, R. Wigand, J. P. Kaltwasser, and M. Loos, "Complete functional C1q deficiency associated with systemic lupus erythematosus (SLE)," *Clinical and Experimental Immunology*, vol. 94, no. 2, pp. 267–272, 1993.

[147] M. J. Walport, K. A. Davies, and M. Botto, "C1q and systemic lupus erythematosus," *Immunobiology*, vol. 199, no. 2, pp. 265–285, 1998.

[148] C. Lood, B. Gullstrand, L. Truedsson et al., "C1q inhibits immune complex–induced interferon-α production in plasmacytoid dendritic cells: a novel link between C1q deficiency and systemic lupus erythematosus pathogenesis," *Arthritis and Rheumatism*, vol. 60, no. 10, pp. 3081–3090, 2009.

[149] M. Son, F. Santiago-Schwarz, Y. Al-Abed, and B. Diamond, "C1q limits dendritic cell differentiation and activation by engaging LAIR-1," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 46, pp. E3160–E3167, 2012.

[150] B. Berghöfer, T. Frommer, G. Haley, L. Fink, G. Bein, and H. Hackstein, "TLR7 ligands induce higher IFN-α production in females," *Journal of Immunology*, vol. 177, no. 4, pp. 2088–2096, 2006.

[151] X. Li, Y. Xu, L. Ma, L. Sun, G. Fu, and Y. Hou, "17beta-estradiol enhances the response of plasmacytoid dendritic cell to CpG," *PloS ONE*, vol. 4, no. 12, Article ID e8412, 2009.

[152] O. Berggren, N. Hagberg, G. Weber, G. V. Alm, L. Rönnblom, and M.-L. Eloranta, "B lymphocytes enhance interferon-α production by plasmacytoid dendritic cells," *Arthritis & Rheumatism*, vol. 64, no. 10, pp. 3409–3419, 2012.

[153] P. Duffau, J. Seneschal, C. Nicco et al., "Platelet CD154 potentiates interferon-α secretion by plasmacytoid dendritic cells in systemic lupus erythematosus," *Science Translational Medicine*, vol. 2, no. 47, Article ID 47ra63, 2010.

[154] N. Hagberg, O. Berggren, D. Leonard et al., "IFN-α production by plasmacytoid dendritic cells stimulated with RNA-containing immune complexes is promoted by NK Cells via MIP-1β and LFA-1," *Journal of Immunology*, vol. 186, no. 9, pp. 5085–5094, 2011.

[155] M.-L. Eloranta, T. Lovgren, D. Finke et al., "Regulation of the interferon-α production induced by RNA-containing immune complexes in plasmacytoid dendritic cells," *Arthritis & Rheumatism*, vol. 60, no. 8, pp. 2418–2427, 2009.

[156] D. M. Santer, B. E. Hall, T. C. George et al., "C1q deficiency leads to the defective suppression of IFN-α in response to nucleoprotein containing immune complexes," *The Journal of Immunology*, vol. 185, no. 8, pp. 4738–4749, 2010.

[157] B. Cederblad, S. Blomberg, H. Vallin, A. Perers, G. V. Alm, and L. Rönnblom, "Patients with systemic lupus erythematosus have reduced numbers of circulating natural interferon-α-producing cells," *Journal of Autoimmunity*, vol. 11, no. 5, pp. 465–470, 1998.

[158] K. Sacre, L. A. Criswell, and J. M. McCune, "Hydroxychloroquine is associated with impaired interferon-alpha and tumor necrosis factor-alpha production by plasmacytoid dendritic cells in systemic lupus erythematosus," *Arthritis Research & Therapy*, vol. 14, no. 3, article R155, 2012.

[159] S.-K. Kwok, J.-Y. Lee, S.-H. Park et al., "Dysfunctional interferon-alpha production by peripheral plasmacytoid dendritic cells upon Toll-like receptor-9 stimulation in patients with systemic lupus erythematosus," *Arthritis Research & Therapy*, vol. 10, no. 2, article R29, 2008.

[160] D. Fabricius, M. Neubauer, B. Mandel et al., "Prostaglandin E_2 inhibits IFN-α secretion and Th1 costimulation by human plasmacytoid dendritic cells via E-prostanoid 2 and E-prostanoid 4 receptor engagement," *The Journal of Immunology*, vol. 184, no. 2, pp. 677–684, 2010.

[161] D. Lindau, J. Mussard, A. Rabsteyn et al., "TLR9 independent interferon alpha production by neutrophils on NETosis in response to circulating chromatin, a key lupus autoantigen," *Annals of the Rheumatic Diseases*, vol. 73, no. 12, pp. 2199–2207, 2014.

[162] M. F. Denny, S. Yalavarthi, W. Zhao et al., "A distinct subset of proinflammatory neutrophils isolated from patients with systemic lupus erythematosus induces vascular damage and synthesizes type I IFNs," *The Journal of Immunology*, vol. 185, no. 6, p. 3779, 2010.

[163] A. Palanichamy, J. W. Bauer, S. Yalavarthi et al., "Neutrophil-mediated IFN activation in the bone marrow alters B cell development in human and murine systemic lupus erythematosus," *Journal of Immunology*, vol. 192, no. 3, pp. 906–918, 2014.

[164] G. McDonald, N. Cabal, A. Vannier et al., "Female bias in systemic lupus erythematosus is associated with the differential expression of X-linked Toll-like receptor 8," *Frontiers in Immunology*, vol. 6, article 457, 2015.

[165] C. Richez, K. Yasuda, A. A. Watkins et al., "TLR4 ligands induce IFN-α production by mouse conventional dendritic cells and human monocytes after IFN-β priming," *The Journal of Immunology*, vol. 182, no. 2, pp. 820–828, 2009.

[166] T. Lövgren, M.-L. Eloranta, B. Kastner, M. Wahren-Herlenius, G. V. Alm, and L. Rönnblom, "Induction of interferon-α by immune complexes or liposomes containing systemic lupus erythematosus autoantigen- and Sjögren's syndrome auto antigen—associated RNA," *Arthritis and Rheumatism*, vol. 54, no. 6, pp. 1917–1927, 2006.

[167] P. Y. Lee, J. S. Weinstein, D. C. Nacionales et al., "A novel type I IFN-producing cell subset in murine lupus," *The Journal of Immunology*, vol. 180, no. 7, pp. 5101–5108, 2008.

[168] J. Klarquist, C. M. Hennies, M. A. Lehn, R. A. Reboulet, S. Feau, and E. M. Janssen, "STING-mediated DNA sensing promotes antitumor and autoimmune responses to dying cells," *The Journal of Immunology*, vol. 193, no. 12, pp. 6124–6134, 2014.

[169] A. E. Wiedeman, D. M. Santer, W. Yan, S. Miescher, F. Käsermann, and K. B. Elkon, "Contrasting mechanisms of interferon-α inhibition by intravenous immunoglobulin after induction by immune complexes versus Toll-like receptor agonists," *Arthritis & Rheumatism*, vol. 65, no. 10, pp. 2713–2723, 2013.

[170] C. Lood, M. Allhorn, R. Lood et al., "IgG glycan hydrolysis by endoglycosidase S diminishes the proinflammatory properties of immune complexes from patients with systemic lupus erythematosus: a possible new treatment?" *Arthritis and Rheumatism*, vol. 64, no. 8, pp. 2698–2706, 2012.

[171] P. Lenert, K. Yasuda, L. Busconi et al., "DNA-like class R inhibitory oligonucleotides (INH-ODNs) preferentially block autoantigen-induced B-cell and dendritic cell activation in vitro and autoantibody production in lupus-prone MRL-Faslpr/lpr mice in vivo," *Arthritis Research & Therapy*, vol. 11, no. 3, article R79, 2009.

[172] M. Hirai, N. Kadowaki, T. Kitawaki et al., "Bortezomib suppresses function and survival of plasmacytoid dendritic cells by targeting intracellular trafficking of Toll-like receptors and endoplasmic reticulum homeostasis," *Blood*, vol. 117, no. 2, pp. 500–509, 2011.

[173] H. T. Ichikawa, T. Conley, T. Muchamuel et al., "Beneficial effect of novel proteasome inhibitors in murine lupus via dual inhibition of type I interferon and autoantibody-secreting cells," *Arthritis and Rheumatism*, vol. 64, no. 2, pp. 493–503, 2012.

[174] H. Amuro, T. Ito, R. Miyamoto et al., "Statins, inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, function as inhibitors of cellular and molecular components involved in type I interferon production," *Arthritis and Rheumatism*, vol. 62, no. 7, pp. 2073–2085, 2010.

[175] V. Salvi, D. Bosisio, S. Mitola, L. Andreoli, A. Tincani, and S. Sozzani, "Trichostatin A blocks type I interferon production by activated plasmacytoid dendritic cells," *Immunobiology*, vol. 215, no. 9-10, pp. 756–761, 2010.

[176] M. Petri, D. J. Wallace, A. Spindler et al., "Sifalimumab, a human anti-interferon-α monoclonal antibody, in systemic lupus erythematosus: a phase I randomized, controlled, dose-escalation study," *Arthritis & Rheumatism*, vol. 65, no. 4, pp. 1011–1021, 2013.

[177] H.-K. Kang, M. Liu, and S. K. Datta, "Low-dose peptide tolerance therapy of lupus generates plasmacytoid dendritic cells that cause expansion of autoantigen-specific regulatory T cells and contraction of inflammatory Th17 cells," *Journal of Immunology*, vol. 178, no. 12, pp. 7849–7858, 2007.

[178] S. R. Christensen, J. Shupe, K. Nickerson, M. Kashgarian, R. Flavell, and M. J. Shlomchik, "Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus," *Immunity*, vol. 25, no. 3, pp. 417–428, 2006.

[179] X. Wu and S. L. Peng, "Toll-like receptor 9 signaling protects against murine lupus," *Arthritis & Rheumatism*, vol. 54, no. 1, pp. 336–342, 2006.

[180] M.-L. Santiago-Raber, I. Dunand-Sauthier, T. Wu et al., "Critical role of TLR7 in the acceleration of systemic lupus erythematosus in TLR9-deficient mice," *Journal of Autoimmunity*, vol. 34, no. 4, pp. 339–348, 2010.

[181] K. M. Nickerson, J. L. Cullen, M. Kashgarian, and M. J. Shlomchik, "Exacerbated autoimmunity in the absence of TLR9 in MRL.*Fas*^{lpr} mice depends on *Ifnar1*," *Journal of Immunology*, vol. 190, no. 8, pp. 3889–3894, 2013.

Hindawi Publishing Corporation
Journal of Immunology Research
Volume 2016, Article ID 9747480, 13 pages
http://dx.doi.org/10.1155/2016/9747480

Research Article

Slight Pro-Inflammatory Immunomodulation Properties of Dendritic Cells by *Gardnerella vaginalis*: The "Invisible Man" of Bacterial Vaginosis?

Thomas Bertran,[1] Patrick Brachet,[2] Marjolaine Vareille-Delarbre,[1] Julie Falenta,[1] Annie Dosgilbert,[3] Marie-Paule Vasson,[4] Christiane Forestier,[5] Arlette Tridon,[1,3] and Bertrand Evrard[1,3]

[1] *UFR Médecine-Pharmacie, Clermont Université, Université d'Auvergne, UMR 1019 UNH, ECREIN, Laboratoire d'Immunologie, 63001 Clermont-Ferrand, France*
[2] *INRA de Theix, UMR 1019 UNH, ECREIN, 63122 Saint-Genès-Champanelle, France*
[3] *Service d'Immunologie, CHU Clermont-Ferrand, 63001 Clermont-Ferrand, France*
[4] *UFR Pharmacie, Clermont Université, Université d'Auvergne, UMR 1019 UNH, ECREIN, Laboratoire de Biochimie, 63001 Clermont-Ferrand, France*
[5] *UFR Pharmacie, Clermont Université, Université d'Auvergne, UMR CNRS 6023 LMGE, Laboratoire de Bactériologie, 63001 Clermont-Ferrand, France*

Correspondence should be addressed to Bertrand Evrard; bevrard@chu-clermontferrand.fr

Received 22 October 2015; Revised 4 January 2016; Accepted 10 January 2016

Academic Editor: Silvia Beatriz Boscardin

Bacterial vaginosis (BV), the most common genital infection in reproductive-aged women, is associated with increased risk of sexually transmitted infections. Its etiology remains unclear, especially the role of *Gardnerella* (*G.*) *vaginalis*, an anaerobic bacterium characteristic of the BV-alteration of the vaginal ecosystem. In the genital mucosa, dendritic cells (DCs) sense bacteria of the microenvironment *via* receptors and then orchestrate the immune response by induction of different T cell subtypes. We investigated the interactions between *G. vaginalis* and human monocyte-derived DCs using a wide range of bacterial concentrations (multiplicity of infection from 0.01 to 100), and the effects of this pathogen on PHA-induced lymphocyte proliferation. As observed by electron microscopy and cytometry, *G. vaginalis* reduced the internalization ability of DCs by forming extracellular clusters and induced neither DC maturation, nor DC secretion of cytokines, except at the highest dose with a very early DC maturation state. The same profile was observed on lymphocytes with significant increases of proliferation and cytokine secretion only at the highest bacterial concentration. Our findings indicate that *G. vaginalis* possesses slight immune-stimulating activities against DCs and T cells, reflecting thus a defective inflammatory response and giving rise to the atypical, non- or low-grade, inflammatory clinical disease profile.

1. Introduction

Bacterial vaginosis (BV) is the most common low genital infection among reproductive-aged women, with a prevalence of 29% among 14- to 49-year-old US women and almost 40% in individuals at high risk for sexually transmitted infections (STIs) [1]. BV is associated with serious medical complications, including adverse pregnancy outcomes, endometritis, and pelvic inflammatory disease such as endometriosis [2, 3]. BV also increases women's risk of acquiring STIs, particularly HIV infections [4, 5].

Clinically, one-half of BV-positive women are asymptomatic while the others suffer only from mild symptoms, such as homogeneous white vaginal discharge and amine (fishy) odor [6]. These signs are associated with a vaginal pH > 4.5 and the presence of characteristic "clue cells" on microscopic examination. These four manifestations constitute Amsel's clinical criteria [6]. The microbiological diagnosis

of BV is usually based on Nugent's score, which includes assessment of lactobacilli by Gram's stain of vaginal fluid samples. BV is associated with an alteration of the vaginal ecosystem, characterized by a decrease in hydrogen peroxide-producing *Lactobacillus (L.)* species such as *L. crispatus* and *L. jensenii* and a concomitant increase in polymicrobial anaerobic bacteria like *Gardnerella* (*G.*) *vaginalis* [7, 8].

The microbial etiology of BV is unclear and a matter of debate [9]. Two opposing hypotheses exist [10]. In the monomicrobial hypothesis, historically the first one, *G. vaginalis* is the single, specific etiologic agent of BV [11]. In the polymicrobial hypothesis, which has gained general acceptance in the last 20 years, *G. vaginalis* acts synergically with other anaerobes to unbalance the vaginal flora and trigger the disease [12]. Vaginal inoculation experiments in the monkey show thus that the co-occurrence of anaerobes and *G. vaginalis* is required to induce BV [13]. Moreover, *G. vaginalis* is frequently isolated in healthy women without BV [14]. Nevertheless, recent works have relaunched the debate by confirming its importance in the pathophysiology of the disease. *G. vaginalis* predominates in vaginal BV-associated biofilms, which are implicated in persistent BV, thus constituting a major factor of resistance to standard treatment [15].

Dendritic cells (DCs) are professional antigen presenting cells (APCs) which, by inducing both tolerance and immunity, are critical for the orchestration of the adaptive immune response [16]. Immature DCs reside in peripheral mucosa, where they sense the microenvironment *via* pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs). PRRs include toll-like receptors (TLRs) and C-type lectin receptors (CLRs) [17]. PRR stimulation triggers a DC maturation process with up-regulation or down-regulation of membrane molecules (CD83, CD86 and HLA-DR, and DC-SIGN and Mannose Receptor, resp.) and cytokine production. DC activation by several PAMPs, via distinct PRRs with antagonistic or synergistic effects, modulates their differentiation, which secondarily determines the polarization of the effector T cell responses, *that is,* the balance between Th1, Th2, Th17, and T regulatory (Treg) subsets [18]. Cytokine production by DCs is an important factor in this process. IL-12 production drives polarization towards Th1 cells, whereas synthesis of IL-1β, IL-6, TGFβ and IL-23, and IL-10 promotes induction of Th17 or Treg cells, respectively [19]. These different stages of the immune response have been recently described in the human genital mucosa. Notably, in both the upper and lower tracts, several DC subsets exist and express specific TLRs, such as TLR-6, TLR-7, and TLR-8, and CLRs, such as langerin and DC-SIGN, and are able to induce different T cell subpopulations [20–22].

The effects of mucosal fluids from women with BV or healthy flora, without analysis of implicated bacterium species, were examined on DC function [23, 24]. BV samples induced IL-12 and IL-23 production, as well as expression of maturation markers (HLA-DR, CD40, and CD83) by monocyte-derived DCs (moDCs). Concerning T cells, there has been yet no investigation on the impact of BV on the polarization of the different lymphocyte subpopulations. Only one study reported effects of BV on the percentage of Treg cells in peripheral blood mononuclear cells (PBMCs). However, this study did not test the possibility of a specific impact of *G. vaginalis* and it did not objectify differences in the distribution of Treg in BV+ *versus* BV− HIV-negative women, decreased Treg being solely observed in BV+/HIV+ women compared to BV−/HIV+ women [25]. Many studies have attempted to measure cervicovaginal production of cytokines in BV, but disparate results were obtained. Most articles reported an elevation in IL-1β, and less consistently in IL-6 and IL-8, in BV-affected women [26–29]. Additionally, BV mucosal fluid was found to increase proliferation of T cells in allogeneic mixed-leukocyte reaction (MLR) [23]. Finally, the specific effects of *G. vaginalis* on DC and T cells have never been evaluated yet.

Unlike conventional vaginitis that is characterized by burning, dysuria, dyspareunia, and frequent pruritus, BV causes scant inflammatory signs without primary pain or pruritus in affected women [14]. Likewise, a relative paucity of inflammatory cells and a near normal number of vaginal neutrophils are characteristic of BV status. In view of the literature data, we hypothesized that BV corresponds to a unique local immunological environment, with a low-grade inflammation, potentially mediated by so far unknown immunomodulatory mechanisms of action of *G. vaginalis* on the vaginal immune system, particularly on the DCs and T cells. In the present study, we investigated this hypothesis in *in vitro* models by monitoring (i) the internalization, maturation, and cytokine secretion of moDCs; (ii) lymphocyte proliferation and subset cytokine production, after cell exposure to *G. vaginalis* or to commensal or pathogenic microorganisms potentially found in the vaginal mucosa.

2. Material and Methods

2.1. Bacterial Strains and Culture Conditions. *G. vaginalis* ATCC14018 was grown in brain heart infusion (Biomérieux) supplemented with maltose (0.1%), glucose (0.1%), yeast extract (1%), and horse serum (10%) in 5% CO_2 at 37°C for 72 h. *L. reuteri* ATCC23272 was grown in De Man, Rogosa, Sharpe (MRS) medium (BD Difco™) at 37°C overnight. *Candida albicans* ATCC10231 was grown in Sabouraud broth at 37°C overnight. Microbial cells were harvested by centrifugation (11,000 ×g for 10 min), and the pellet was washed twice and then resuspended in RPMI 1640 (Cambrex Bio Science). Optical density (OD) measurements were performed at 620 nm to adjust the final concentration of the microbial suspension, and the exact number of colony forming units (CFU) was determined by plating serial dilutions of the inocula onto adapted agar plates (Columbia 5% Sheep Blood Agar, MRS, or Sabouraud). Before being added to the cell samples, the microbial cells were inactivated by exposure to UV for 1 h. The effectiveness of the inactivation was evaluated by plating 20 μL of the irradiated inocula on adapted agar plates.

2.2. Ethics Statement. The human cells used in this study were generated from the buffy-coat of healthy volunteers obtained

from the local French blood agency (Etablissement Français du Sang, EFS, Saint-Etienne). It is a statutory requirement that blood donors be given full necessary information (article R.1221-5 of the Public Health Code, 12/01/2009 and 11/06/2006 decrees). Written informed consent was obtained by the EFS from all volunteers involved in our study.

2.3. In Vitro Differentiation of Monocyte-Derived Dendritic Cells. DCs were generated from PBMCs. Briefly, PBMCs were isolated from the buffy-coats of healthy volunteers by Ficoll-Histopaque (Sigma) density gradient centrifugation. PBMCs were washed twice in RPMI 1640 and resuspended at a final concentration of 5×10^7 cells per mL of phosphate buffered saline (PBS) supplemented with 2% fetal calf serum (FCS, Biowest-Abcys) and 1 mM EDTA. Monocytes were purified by negative selection using the EasySep$^{\text{Ŷ}}$ Human Monocyte Enrichment Kit, as recommended by the manufacturer (StemCell Technologies). They were then cultured for 5 days in RPMI 1640 supplemented with 1% L-glutamine (Sigma), 10% FCS, and 0.5% penicillin-streptomycin (Sigma), in the presence of 500 U/mL IL4 (R&D systems) and 800 U/mL granulocyte-macrophage colony-stimulating factor (GM-CSF, R&D systems). After 3 days of incubation, one-half volume of fresh culture medium containing 2x concentrations of IL4 and GM-CSF was added to each well.

2.4. Electron Microscope Observations. DCs obtained as previously described were plated in a sterile 12-well plate at a concentration of 1×10^6 cells/mL. *G. vaginalis* was added to wells at a multiplicity of infection (MOI) of 10 for 1 h (Scanning Electron Microscopy, SEM) and at a MOI of 0.01, 1, or 100 for 3 h (Transmission Electron Microscopy, TEM). Cells were harvested, centrifuged (400 ×g for 10 min), rinsed with Natrium Cacodylate (0.2 M pH 7.4) for 10 min, and then fixed at 4°C overnight with glutaraldehyde 1.6% in Natrium Cacodylate buffer. The samples were then rinsed, postfixed with 1% Osmium Tetroxide (1 h, room temperature), rinsed again, and dehydrated with graded series of ethanol (70 to 100%) and eventually with 100% hexamethyldisilazane. Finally, after overnight drying, samples were placed on a Jeol SEM filter and metallised with carbon (40 s). For TEM, dried samples were embedded in a polymerized 2 mm thick Epon coating, and ultrathin sections were picked up with Formvar-coated copper grids (300 mesh). Sections were counterstained with 4% aqueous uranyl acetate. For negative staining, bacteria were grown overnight in M63B1-0.4% Glu medium and negatively stained with 2% phosphotungstic acid on Formvar-coated copper grids (300 mesh). Images were captured at the Centre d'Imagerie Cellulaire Santé (CICS) of the Université d'Auvergne with a Jeol JSM-6060LV (SEM) and a Hitachi H-7650 (TEM).

2.5. Flow Cytometry Analysis of DC Maturation and Viability. On day 6, the immature DCs from each well were harvested, pooled, centrifuged, and reseeded at 1×10^5 cells/mL. UV-killed bacteria were then added at 10 μL of suspension per well to reach a final concentration ranging from 10^3 to 10^7 CFU/mL, *that is,* a MOI between 0.01 and 100. Lipopolysaccharide (LPS) from *Escherichia coli* (Sigma) at a final concentration of 100 ng/mL was used as positive control. Immature DCs without addition of LPS or bacteria were used as negative control. After 48 h of maturation at 37°C in a 5% CO_2 atmosphere, DCs were collected, centrifuged, and resuspended in PBS with 1% bovine serum albumin (BSA, Sigma). Cell surfaces were stained with the appropriate fluorescence-labeled murine antibodies: APC-Cy7-conjugated anti-CD14 (LPS coreceptor specific to monocytes), PE-conjugated anti-CD86 (costimulatory molecule, activation marker), V450-conjugated anti-HLA-DR, PerCP-Cy5.5-conjugated anti-DC-SIGN (DC-specific ICAM-3 grabbing-nonintegrin or CD209, member of the CLR family, specific marker of immature DCs), Alexa Fluor$^{\text{Ŷ}}$ 488-conjugated anti-MR (Mannose Receptor or CD206, member of the CLR family, specific marker of immature DCs and macrophages), and streptavidin APC-conjugated anti-TLR4 (biotin antibody, an LPS receptor with activation functions). Antibodies were obtained from BD Biosciences, except anti-MR (Biolegend). Corresponding murine isotype-matched and non-labeled antibodies (BD Biosciences or Biolegend) were used as controls. The cells were analyzed by a BD-LSRII flow cytometer with FACSDiva Software (BD Biosciences) at the CICS. Fluorescence compensation adjustments were performed. Gates were set on living DCs based on their forward/side scatter (FSC/SSC) properties. The analysis was halted at a count of 3,000 DCs. The level of staining was expressed as the mean fluorescence intensity (MFI). Culture supernatants were collected and stored at −20°C until cytokine analysis. To determine cell viability, dye LIVE/DEAD$^{\text{Ŷ}}$ beads (Fixable Blue Dead Cell Stain Kit, for UV excitation Life technologies) were added to cells and used as markers of dead cells. DC mortality was assessed by gating together dead cells and live DCs on SSC/FSC diagram. Two other gates were created from this gate to separate the two populations. From the dead cell gate, cells that expressed LIVE/DEAD marker were considered as effectively dead. This dead cell number was expressed as a ratio of the first created overall gate to obtain a dead DC percentage.

2.6. Lymphocyte Proliferation Assays. The mitogenic response to plant lectins, as phytohemagglutinin A (PHA), is conventionally used to measure cell-mediated immunity in mammals in general and especially in humans [30]. These tests are named lymphocyte proliferation assays or lymphocyte transformation test (LTT). They were performed here to study the functional properties of *G. vaginalis* and especially its capabilities to modulate PHA-induced T cell proliferation. On day 1, PBMCs collected from a buffy-coat as previously described were adjusted to a concentration of 1×10^6 cells per mL of complete medium, *that is,* RPMI 1640 supplemented with 1% L-glutamine and 10% FCS. The cell suspension was deposited in a sterile 96-well plate (100 μL per well). Each measurement was done in triplicate. Polyclonal proliferation of lymphocytes was induced with 2 μg/mL of PHA (Sigma). Cells without PHA or bacteria were used as a negative control and cells with PHA without bacteria as a positive one. *G. vaginalis* was added to other PHA-treated wells

to obtain concentrations ranging from 10^3 to 10^7 CFU/mL. After a 72 h incubation at 37°C under 5% CO_2, 1 μCi of tritiated thymidine (Perkin Elmer) was added to each well and the cells were further incubated for 4 h. Labeling was stopped by cooling the plate to 4°C. The cells were then collected under vacuum onto a Whatman filter paper and incorporation of tritiated thymidine was measured using a β counter (Tri-Carb 2300TR, Canberra-Packard). Proliferation results were expressed as mean cpm (counts per min) values of triplicate measurements. Identical incubations, however, without addition of tritiated thymidine, were carried out in parallel to collect supernatants for cytokine quantification.

2.7. Cytokine Quantification. For DC maturation experiments, the cytokines IL-10, TNF-α, IFN-γ, and IL-12p70 were assayed in culture supernatants with Biolegend enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. For lymphocyte proliferation assays, the cytokines IFN-γ, IL-4, IL-17A, IL-10, IL-12p70, and TNF-α were quantified in supernatants of PBMC cultures using Pro Human Cytokine Group 1 6-Plex 1 × 96 kit (Bio-Rad) on a Bio Plex$^{\text{Ý}}$ 200 (Bio-Rad).

2.8. Statistical Analysis. All data were expressed as means + SD. After variance dispersion test, three different statistical tests were performed. Two-way ANOVA with *post hoc* Bonferroni test, Friedman's test with Nemenyi's group, and Kruskal-Wallis test with Dunn test were used to analyze the significant effect of bacteria on DCs or PBMCs with XLStat 7.5.2 software (Addinsoft, Paris, France). p values lower than 0.05 were considered statistically significant.

3. Results

3.1. Electron Microscopy Observation of G. vaginalis-DC Interaction. *In vivo*, the first step of the immune response in the vaginal mucosa corresponds to the interactions between bacteria and immature DCs, which lead to the internalization of bacteria. To mimic this initial phenomenon *in vitro,* we put in contact DCs with *G. vaginalis* during 1 to 3 h and took SEM and TEM pictures. SEM produced 3D images of cell and bacteria surfaces after 1 h of contact (Figures 1(a) and 1(b)). *G. vaginalis* bacteria were rarely found on isolated cell forms (in contrast with what we observed with *Lactobacillus,* data not shown) and were in general organized in clusters (Figure 1(a)). However, DC dendrites interacted with this cluster of bacteria and sometimes surrounded it (Figure 1(b)). TEM realized at 3 h, to allow the cells to have time to internalize bacteria, confirmed interactions of DC dendrites with clusters of *G. vaginalis* (Figures 1(c) and 1(d)). Figure 1(c) shows additionally that the cluster is composed of bacteria enrobed in an extracellular matrix which may interact with DCs. TEM also shows internalized intracellular *G. vaginalis* bacteria, but only in their isolated form, without intracellular clusters (Figure 1(e)). At a MOI of 0.01 no DCs with internalized *G. vaginalis* were found, very few at a MOI of 1, and only 35% at a MOI of 100 (count on 100 DCs). For each DC with internalized *G. vaginalis*, the number of bacteria ranged from 1 to 9 bacteria per cell. This experiment was performed in parallel with *L. reuteri* at the same concentrations. Internalization of *Lactobacillus*, unlike *G. vaginalis*, could be observed on few cells at a MOI of 0.01, a majority of cells at a MOI of 1, and up to 82% of cells at a MOI of 100 (count on over 160 DCs). The number of *Lactobacillus* internalized in those cells was higher than with that of *G. vaginalis* since it ranged from 6 to 18 bacteria per cell (data not shown). To conclude on this part, we showed that DCs were able to interact with *G. vaginalis* and to internalize it, but less effectively than lactobacilli, probably due to *G. vaginalis* ability to form extracellular clusters.

3.2. Flow Cytometric Analysis of Microbial Effects on DC Phenotype. During the immune response, bacterium internalization can then induce DC activation and maturation, resulting in modifications detectable by cytometry of numerous membrane markers and permitting to distinguish immature DCs from mature ones. In our study, DC activation and maturation were assessed by changes affecting an extensive phenotype of the cell membrane. As expected, immature DCs were characterized by low levels of CD86, HLA-DR, and CD14 expression (the latter was compared to its initial level in monocytes before IL-4 and GM-CSF treatment, data not shown), together with high levels of DC-SIGN and MR expression (Figures 2 and 3). Comparatively, LPS-induced DCs expressed a phenotype characteristic of fully mature DCs with increased levels of CD86 and HLA-DR (Figures 3(a) and 3(b)) associated with decreased levels of DC-SIGN, MR, TLR4, and CD14 (Figures 3(c), 3(d), 3(e), and 3(f)).

Incubation with low or medium doses of *G. vaginalis* (10^3–10^6 CFU/mL, *i.e.,* MOI from 0.01 to 10) did not alter immature DC cell surface phenotype, as shown by the absence of significant change in either membrane marker (Figures 2 and 3). At the highest concentration (10^7 CFU/mL, *i.e.,* MOI 100), *G. vaginalis* caused a very slight increase in HLA-DR expression (statistically non-significant, Figure 3(b)) combined with moderate decreases in MR and CD14 expressions (both non-significant, Figures 3(e) and 3(f)), which reflect early signs of maturation. However, the lack of increase in CD86 expression and decrease in DC-SIGN and TLR4 expressions indicated that this maturation process was incomplete.

Taken together, our overall cytometric results indicate that *G. vaginalis* induced no maturation of DCs or an incomplete DC maturation process at high doses.

Unlike *G. vaginalis*, *L. reuteri* and *C. albicans* induced a clear dose-dependent higher expression of CD86 and HLA-DR upon maturation of DCs (Figures 3(a) and 3(b)). Combined with decreased expressions of DC-SIGN, MR, CD14, and TLR4 (Figures 3(c), 3(d), 3(e), and 3(f)), these results show that the last two microorganisms induced fully mature DCs, similarly to LPS extract.

3.3. Flow Cytometric Analysis of G. vaginalis Effects on DC Mortality. Given the very low level of maturation of DC by *G. vaginalis* observed in our experiments, we hypothesized that this strain would not induce DC cytotoxicity. To test

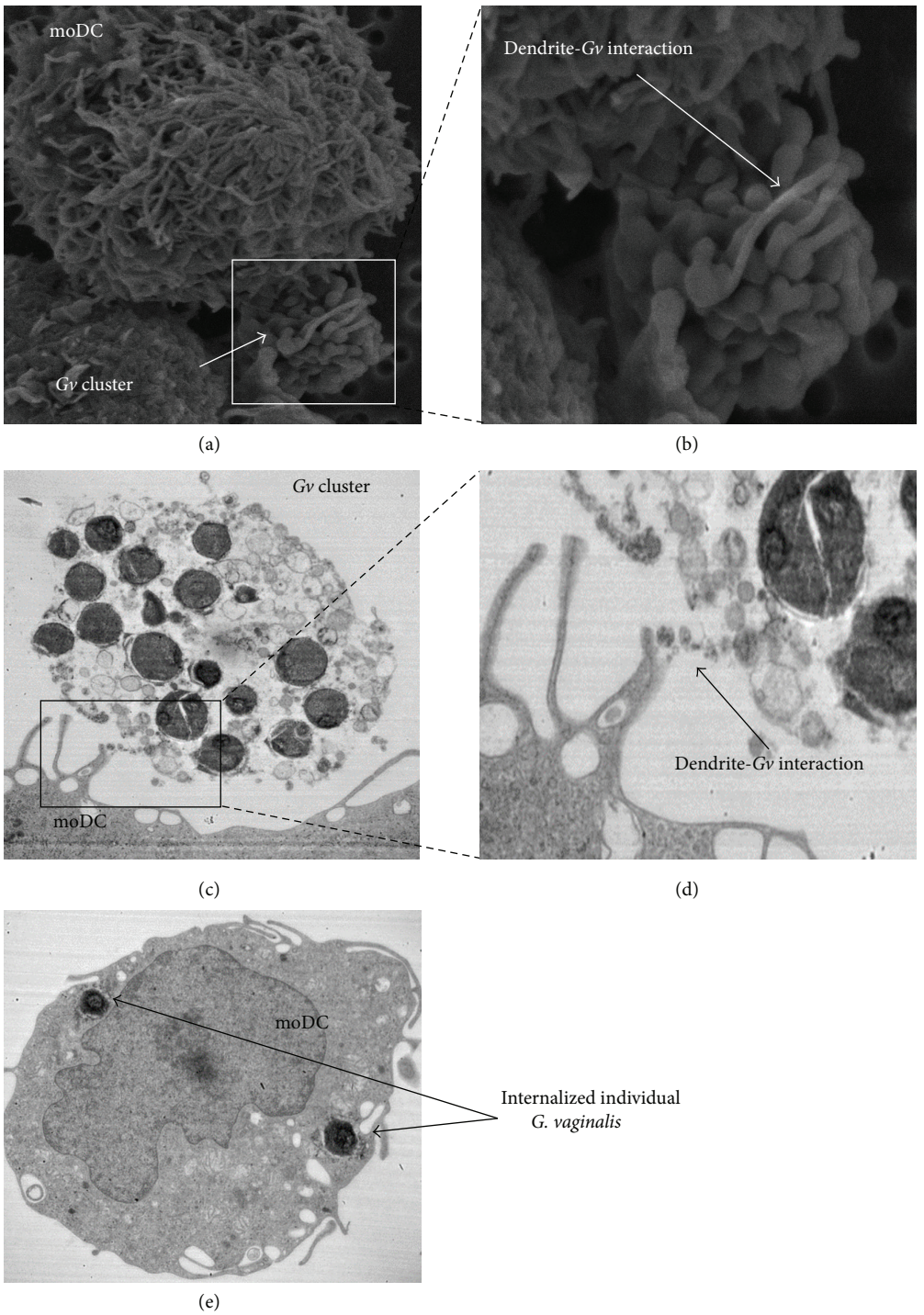


Figure 1: Pictures of DCs after exposure to *G. vaginalis*. (a) SEM (×5500), after a 1 h exposure, DC dendrites surrounding clusters of *G. vaginalis*. (b) Zoom (×13000) on the contact zone between DCs and bacteria. (c) TEM (×12000), after a 3 h exposure, a cluster of *G. vaginalis* in contact with DC dendrites. (d) Enlargement of DC dendrite-*G. vaginalis* interaction zone. (e) TEM cutting, internalized *G. vaginalis* in one DC. moDC: monocyte-derived dendritic cell, *Gv: Gardnerella vaginalis*.

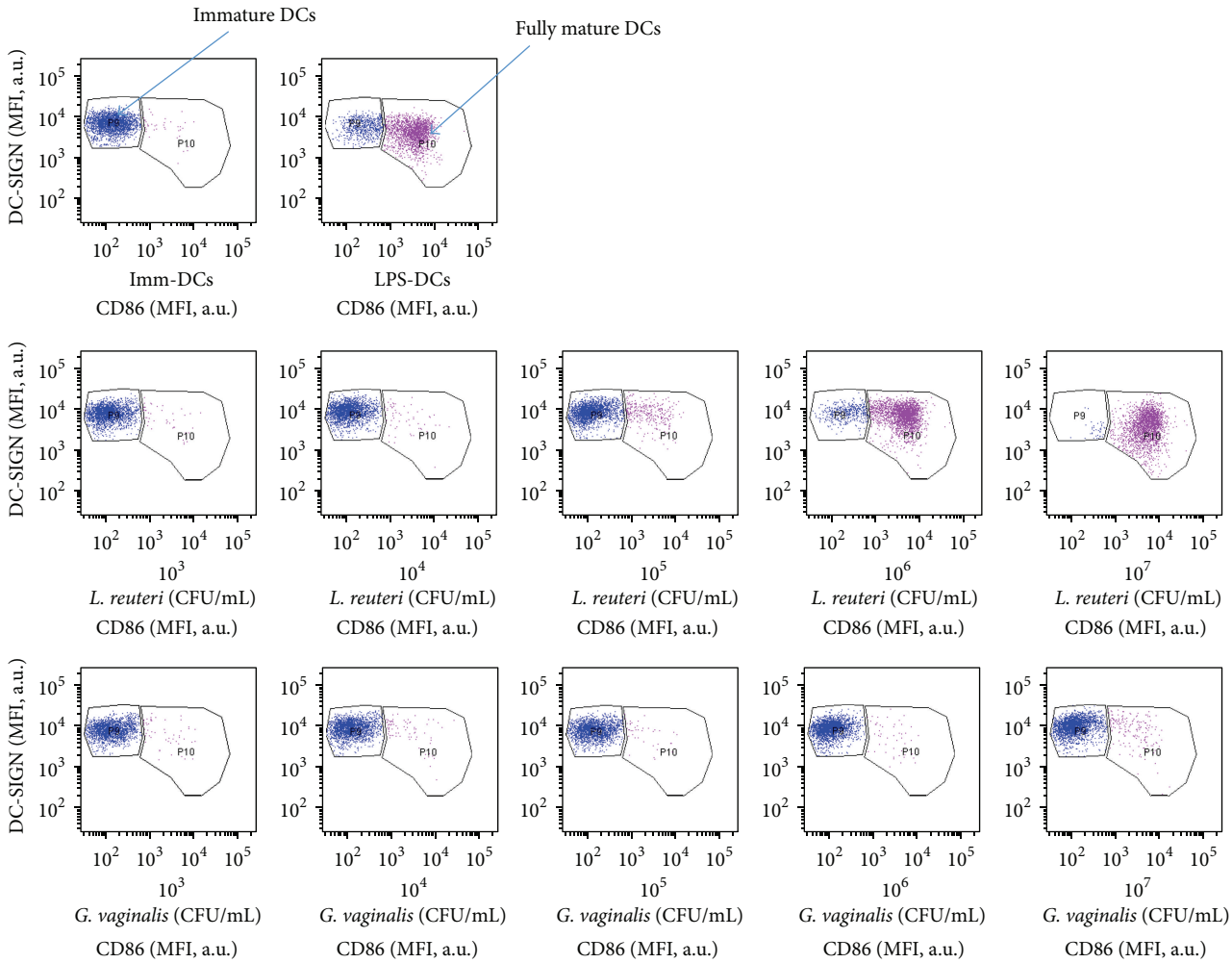


Figure 2: Surface phenotype of human DCs after exposure to a range of *G. vaginalis* or *L. reuteri* concentrations. The dot plots and histograms show MFI values on gated DCs. DC-SIGN/CD86 dot plots gated on human DCs. Data from a representative experiment comparing *G. vaginalis*-induced DC process of maturation with that induced by *L. reuteri*.

this assertion, a viability marker was added during flow cytometric experiments. Immature DCs alone had almost no mortality. In comparison, exposure of DCs to LPS extract induced a slight, but significant, increase in mortality (Figure 4). Three concentrations of *G. vaginalis* were tested to analyze their impact on DC survival**.** Results showed that, from 10^3 to 10^7 CFU/mL (MOI 0.01 to 100), *G. vaginalis* induced no increase in dead DCs.

3.4. Cytokine Secretion by Microbial-Matured DCs. In vivo, mature DCs migrate to secondary lymphoid organs and present the antigen to T cells. During this interaction, DCs deliver three signals, notably a cytokinic signal polarizing the differentiation of lymphocytes into several subpopulations. To decipher the preferential pathway of T cell polarization induced by *G. vaginalis,* four cytokines assumed to be of particular interest were selected for ELISA measurements. Compared to untreated immature DCs, DCs incubated with *G. vaginalis* did not significantly increase the production of TNF-α or IL-10, except at the upper dose of 10^7 CFU/mL (Figures 5(a) and 5(b)). Additionally, the production of IL-12p70 and IFN-γ was not or barely detectable in *G. vaginalis*-treated or immature DCs (Figures 5(c) and 5(d)). Conversely, a strong dose-dependent increase in the production of TNF-α and IL-10 was induced by *L. reuteri* and *C. albicans* and, to a lesser extent, of IL-12p70 by *L. reuteri* and of IFN-γ by *C. albicans* (Figures 5(a), 5(b), 5(c), and 5(d)). The fold changes determined by comparing the level of cytokines produced by DCs exposed to 10^7 CFU/mL *G. vaginalis* (*i.e.,* a MOI of 100) to that produced by immature DCs were equal to 8.9 for IL-10, 6.5 for TNF-α, and only 1.1 and 1.3 for IL-12p70 and IFN-γ, respectively. By comparison, the fold changes induced by the same dose of *L. reuteri* or *C. albicans* ranged from 70 to 307 for IL-10 and TNF-α production. Whatever the concentration of *G. vaginalis*, the level of DC-produced cytokines attained only 10 to 28% of that generated by LPS-treated DCs. Overall, these results show that *G. vaginalis* barely induces cytokinic secretion, in accordance with our findings on DC maturation (Figure 3), thus indicating absence, or slight induction, of DC activation by *G. vaginalis*.

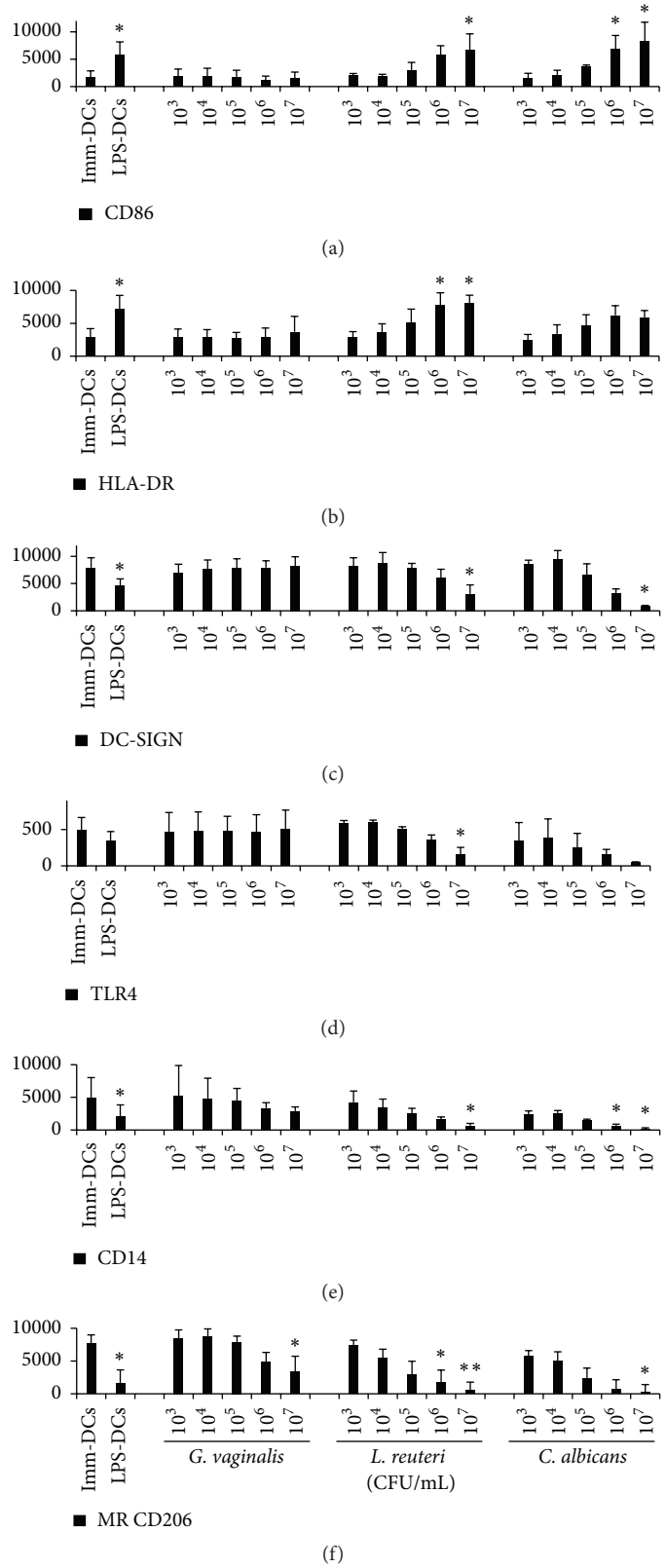


Figure 3: Human DC membrane marker expression after exposure to a range of *G. vaginalis, L. reuteri,* or *C. albicans* concentrations. (a, b) Differential expression of CD86 and HLA-DR, two membrane markers typically increasing during DC maturation. (c, d, e, f) Differential expression of DC-SIGN, TLR4, CD14, and MR CD206, four membrane markers typically decreasing during DC maturation. Data are means (+ SD). For every marker, the isotypic control values were subtracted from the MFI values. Imm-DCs: immature DCs, LPS-DCs: DCs matured by a 48 h exposure to 100 ng/mL *E. coli* LPS; *G. vaginalis*, *L. reuteri*, and *C. albicans*: DCs matured after a 48 h incubation at different concentrations (10^3 to 10^7 CFU/mL, *i.e.,* MOI = 0.01 to 100) of *G. vaginalis* ($n = 5$), *L. reuteri* ($n = 3$), and *C. albicans* ($n = 2$). Kruskal-Wallis test with Dunn comparison. $^* p < 0.05$, $^{**} p < 0.01$, as compared to the Imm-DCs.

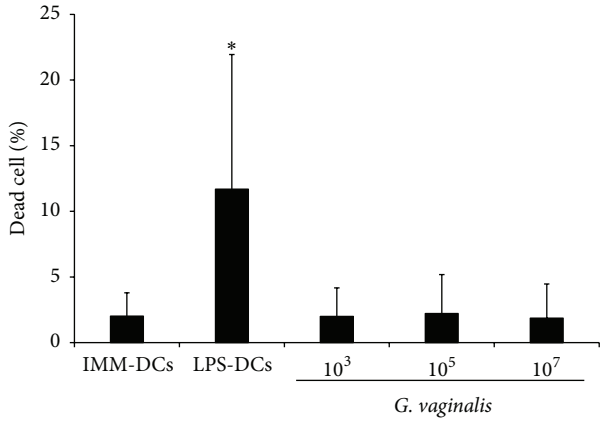


Figure 4: DC viability after 48 h of culture with *G. vaginalis*. Histogram shows the dead cell percentage, calculated from SSC/FSC diagram and live/dead marker. LPS induced a significant increase in DC mortality compared to immature DCs alone in the medium. Low and high concentrations of *G. vaginalis* did not induce any increase in DC mortality, even at a MOI of 100. Data are means (+ SD) from 6 individual experiments. Imm-DCs: immature DCs, LPS-DCs: DCs matured by a 48 h exposure to 100 ng/mL *E. coli* LPS; *G. vaginalis*. DCs matured after a 48 h incubation at different concentrations (10^3 to 10^7 CFU/mL, *i.e.,* MOI = 0.01 to 100) of *G. vaginalis*. Friedman's test with Nemenyi's comparison $^*p < 0.05$.

3.5. Slight Increase in PHA-Stimulated Lymphocyte Proliferation by G. vaginalis. To investigate lymphocyte activation, the next classical stage of the immune response, we carried out functional tests of bacterium-induced modulation of lymphocytic proliferation, using a model of PHA-induced T cell proliferation. Addition of the strain to the medium caused a slight dose-dependent increase in PHA-stimulated lymphocyte proliferation in comparison to lymphocyte control assays without bacteria (Figure 6). The increase in proliferation was only significant at a dose of 10^7 CFU/mL ($p < 0.001$) and attained an upper average value of 20% compared to that of PHA-stimulated control cells. Lower concentrations of *G. vaginalis* did not cause any significant modulation of PHA-stimulated lymphocyte proliferation. Similar experiments we performed in parallel showed that *L. reuteri* and *C. albicans* did not induce a similar pattern (data not shown).

3.6. G. vaginalis-Dependent Increase in Cytokine Secretion by PHA-Stimulated Leukocytes. To depict the type of immune response involved during the *G. vaginalis*-dependent increase in PHA-stimulated lymphocyte proliferation, secretion of cytokines by the four main subpopulations of T cells (IFN-γ, IL-4, IL-17A, and IL-10 corresponding to Th1, Th2, Th17, or Treg, resp.) or by APCs (IL-12p70 and TNF-α) was measured in extracellular media of lymphocyte proliferation assays. PHA (2 μg/mL) alone induced a strong secretion of IFN-γ, IL-17A, IL-10, IL-12p70, and TNF-α from PBMCs (Figure 7). A clear dose-dependent increase in IFN-γ and IL-17A production was observed in PHA-stimulated PBMCs exposed to varying concentrations of *G. vaginalis* (Figures 7(c) and 7(d)). As shown previously for PHA-stimulated proliferation, only the highest dose of *G. vaginalis* tested induced a significant augmentation in the production of these two cytokines, compared to the PHA control without bacteria. For TNF-α, IL-12p70, and IL-10, the increases were non-significant, even at high doses (Figures 7(a), 7(b), and 7(e)). Compared to the control conditions without bacteria, exposure to 10^7 CFU/mL *G. vaginalis* induced fold increases of 4.9, 4.0, 3.0, 2.2, and 2.2 for IFN-γ, IL-10, TNF-α, IL-17A, and IL-12p70 production, respectively. Contrastingly, the secretion level of IL-4 was barely measurable or undetectable at any dose of *G. vaginalis* (not shown). Furthermore, at their highest concentration, *L. reuteri* and *C. albicans* caused an increase in cytokine secretion that, depending on the cytokine, was 4- to 5-fold higher than that caused by *G. vaginalis* (data not shown). Overall, these data show that *G. vaginalis* can induce a slight dose-dependent secretion of cytokines on both the inflammatory (IFN-γ, TNF-α, IL-17A, and IL-12p70) and anti-inflammatory (IL-10) sides.

4. Discussion

DCs, the main sentinels of the immune system, are abundant in the human vaginal mucosa, both in the epithelium and in the lamina propria [21]. In this mucosal area, DCs can interact with luminal microorganisms, either indirectly *via* epithelial transport mechanisms or directly *via* dendrites extended across epithelial cells that take up bacteria from the vaginal lumen [31, 32]. As BV is characterized by an imbalance of the normal H_2O_2-producing *Lactobacillus* flora toward a polymorphic anaerobic flora with predominant *G. vaginalis*, it is likely that the PRRs of vaginal DCs are subsequently affected by this alteration. To gain insight into the pathophysiological role of *G. vaginalis* in BV, we decided to characterize the interactions between this bacterium and DCs.

We first carried out an electron microscope study of bacteria-DC interactions, the first stage in the immune response, to determine whether *G. vaginalis* can internalize DCs. This was confirmed by using TEM, but we observed that the bacteria were very sparsely represented in free form in culture medium and rather formed clusters coated in an extracellular matrix. As compared to a *Lactobacillus* species which remained in free form, *G. vaginalis* was internalized by DCs much less efficiently. It can reasonably be assumed that this difference is related to the propensity of *G. vaginalis* to form clusters. In addition, *G. vaginalis* forms, *in vivo*, biofilms in the vagina, a process that is involved in BV pathogenesis [33]. These clusters might be the beginning of biofilm formation. Biofilms reduce the host-immune response by decreasing bacteria internalization owing to their large size and to the fact that their extracellular matrix can prevent antigen recognition by APCs [34]. This conformation might thus allow bacteria like *G. vaginalis* to be less internalized by DCs comparatively to strains like *Lactobacillus* that are unable to form biofilms.

We then studied the impact of internalization of *G. vaginalis* on DC maturation status. The maturation state of DCs is a critical determinant of the balance between their

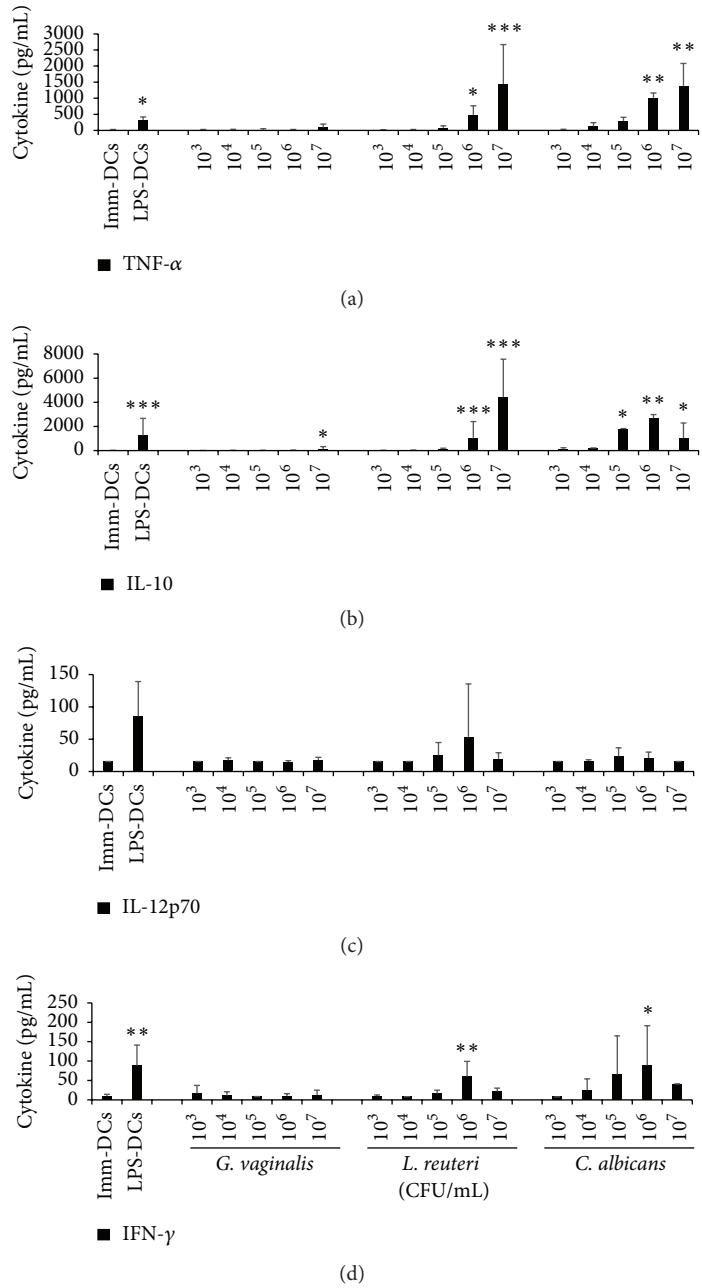


Figure 5: Cytokine production by human DCs exposed to a range of *G. vaginalis, L. reuteri,* or *C. albicans* concentrations. (a, b) TNF-α and IL-10 cytokine production. *G. vaginalis* induceda slight increase in IL-10 secretion at high doses but no significant production of TNF-α. (c, d) IL-12p70 and IFN-γ cytokine production. *G. vaginalis* induced no significant production of the 2 cytokines, even at the highest bacteria concentrations. Data are means (+ SD) of measurements from 6 independent experiments, except for *C. albicans* (3 experiments). Imm-DCs: immature DCs; LPS-DCs: DCs matured by a 48 h exposure to 100 ng/mL *E. coli* LPS; *G. vaginalis*, *L. reuteri*, and *C. albicans*: DCs matured after a 48 h incubation at different concentrations (10^3 to 10^7 CFU/mL, *i.e.,* MOI = 0.01 to 100) of *G. vaginalis*, *L. reuteri,* and *C. albicans*, respectively. Friedman's test with Nemenyi's comparison $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ as compared to the Imm-DCs.

tolerogenic and immunogenic abilities [35]. Immature or semi-mature DCs generally promote tolerogenic responses whereas mature DCs promote immunogenic responses [36]. Flow cytometry analysis showed that, whatever bacterial concentrations, *G. vaginalis* elicited minimal changes in the DC membrane phenotype, thus inducing a very incomplete maturation of human DCs. Concurrently, cytokine production remained very low compared to that from LPS-induced fully mature DCs or *L. reuteri*- or *C. albicans*-matured DCs, even at high doses of microorganisms. Taken together, our cytometric and cytokinic data characterize a non-inflammatory DC response at low *G. vaginalis* doses and a very slight pro-inflammatory DC response at the highest concentration. *G. vaginalis* concentrations interacting

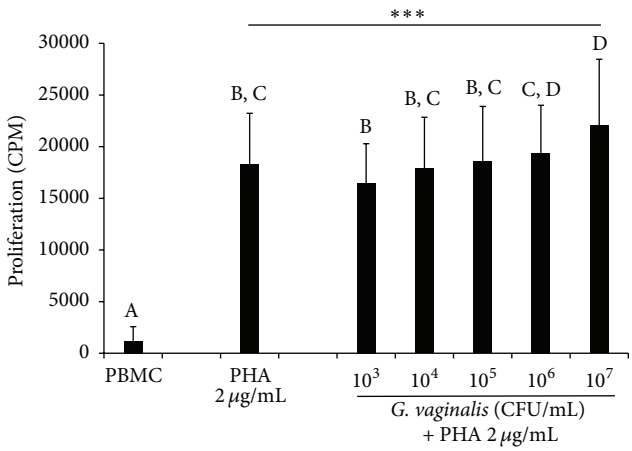


Figure 6: Modulation of PHA-induced lymphocyte proliferation after exposure to a range of *G. vaginalis* concentrations.As expected, 2 μg/mL PHA induced a strong proliferation of the lymphocyte control cells, reaching on average about 20 000 cpm. Compared to the PHA-stimulated control cells, a significant slight increase in lymphocyte proliferation was observed with the *G. vaginalis*-treated cells at a high dose. Data are means (+ SD) of measures from 13 independent experiments. PBMC: PBMCs cultured for 72 h without any effector; PHA control: PBMCs only exposed to PHA (2 μg/mL) for 72 h; *G. vaginalis*: PBMCs exposed for 72 h to PHA (2 μg/mL) in the presence of 10^3 to 10^7 CFU/mL of *G. vaginalis*. Two-way ANOVA with *post hoc* Bonferroni test ($^{***}p < 0.001$). A, B, C, and D: means with different superscript letters are significantly different from each other.

in vivo with mucosal DCs in the human genital tract have not been widely assessed. However, in women with BV, the number of bacteria present can be equal or superior to 10^8 per mL of vaginal fluid, including about 10^7 CFU/mL of *G. vaginalis* [37, 38]. Although unknown, the actual number of bacteria in direct contact with vaginal DCs within the mucosa is certainly much lower than this number. Thus, the concentrations of *G. vaginalis* interacting with vaginal DCs in the mucosa of women with BV probably correspond to the low or intermediate MOIs used in our model and causing no or very little DC maturation. The results obtained with *G. vaginalis* were compared to those obtained with two other microorganisms potentially present in the vaginal mucosa, a commensal bacterium (*L. reuteri*) and a pathogenic yeast responsible for mycotic vaginitis (*C. albicans*). Each induced a clear-cut maturation of the DCs, similar to that we previously observed with other pathogens and probiotic strains [39, 40] but in strong contrast to the *G. vaginalis* DC response. In our model, *G. vaginalis* did not induce DC mortality, unlike the two other microorganisms (data not shown). Overall, our findings show that *G. vaginalis* is slightly pro-inflammatory, but less than the *Lactobacillus* strain we used as control.

The effects of *G. vaginalis* on DCs observed in our study were slighter than those reported in other studies, which showed activation and maturation of human moDCs when exposed to the mucosal fluid of women with BV [23, 24]. In these previous studies, DCs were exposed to a mixture of numerous bacterial products secreted by the characteristic polymorphic BV flora and to molecules produced by the mucosal immune system of women with BV. By contrast, in our model, DCs were placed in the presence of *G. vaginalis* alone to find new evidence of its role as a putative BV etiological agent. Thus, the absence of or the very slight *G. vaginalis*-induced DC maturation by *in vitro* direct contact is not inconsistent with a DC maturation induced by substantially secreted products of the overall BV flora, like, for example, the LPS of *Prevotella bivia*. In the vaginal mucosa, *G. vaginalis* could have local specific modulatory effects on immune cells, independently of an overall effect on the maturation of BV flora.

We next performed functional tests of lymphocytic proliferation using a model of PHA-stimulated PBMCs to investigate the activation of lymphocytes, the following stage in the classical immune response. We observed no significant variation in lymphocyte proliferation at all doses of *G. vaginalis* that we used, except the highest one where a significant increase was measured. These findings are consistent with the variations observed in DC status and confirm that *G. vaginalis* induces very few immunologic effects at low doses and a slight pro-inflammatory response at the highest concentrations, which are unlikely to be encountered *in vivo*. This profile of the immunological response to *G. vaginalis* could explain the characteristic lack of external inflammatory signs during BV, in contrast with bacterial or mycotic vaginitis, despite increased pro-inflammatory TLR2 and TLR4 signaling and IL-1β secretion reported elsewhere [41–43]. In light of our results, we hypothesize that, depending on its actual amount in contact with the mucosal immune cells, *G. vaginalis* could either go unnoticed or induce low-grade inflammation *in vivo*.

To explore the mechanism of the slight *G. vaginalis*-induced increase in lymphocyte proliferation, a large panel of cytokines was measured in the cell supernatants including molecules secreted by APCs (IL-10, IL-12p70, and TNF-α) and/or by T cells (IFN-γ, IL-4, IL-17A, and IL-10). We evidenced a similar *G. vaginalis* dose-dependent profile of secretion for all these cytokines, except for IL-4, which remained undetectable. The higher the dose of the pathogen was, the stronger the PHA-stimulation of cytokine secretion was. Thus, this cytokine secretion profile is clearly related to that of the proliferation of PHA-stimulated PBMCs, irrespective of the anti- or pro-inflammatory nature of the cytokines. With regard to the response to *G. vaginalis* of the four main subpopulations of T cells, cytokine secretion suggests a clear dose-dependent induction of Th1 (IFN-γ, IL-12p70) and Th17 (IL-17A) and Tregs (IL-10), but not of Th2 (IL-4). Thus, Th1, Th17, and Tregs could be involved in the immune response to high doses of *G. vaginalis*. This topic deserves further investigation in future studies of T cell polarization.

Taken together, our findings show that *G. vaginalis*, by forming clusters and reducing the internalizing ability of DC, induces a slight immunological host response including maturation of DCs, lymphocyte proliferation, and pro-Th1, pro-Th17, and pro-Tregs cytokine production. These immunomodulatory properties are consistent with the atypical clinical profile of BV, which is characterized by a low-grade inflammatory process, as we observed in our *in vitro* model at the highest dose of the bacterium. Our results

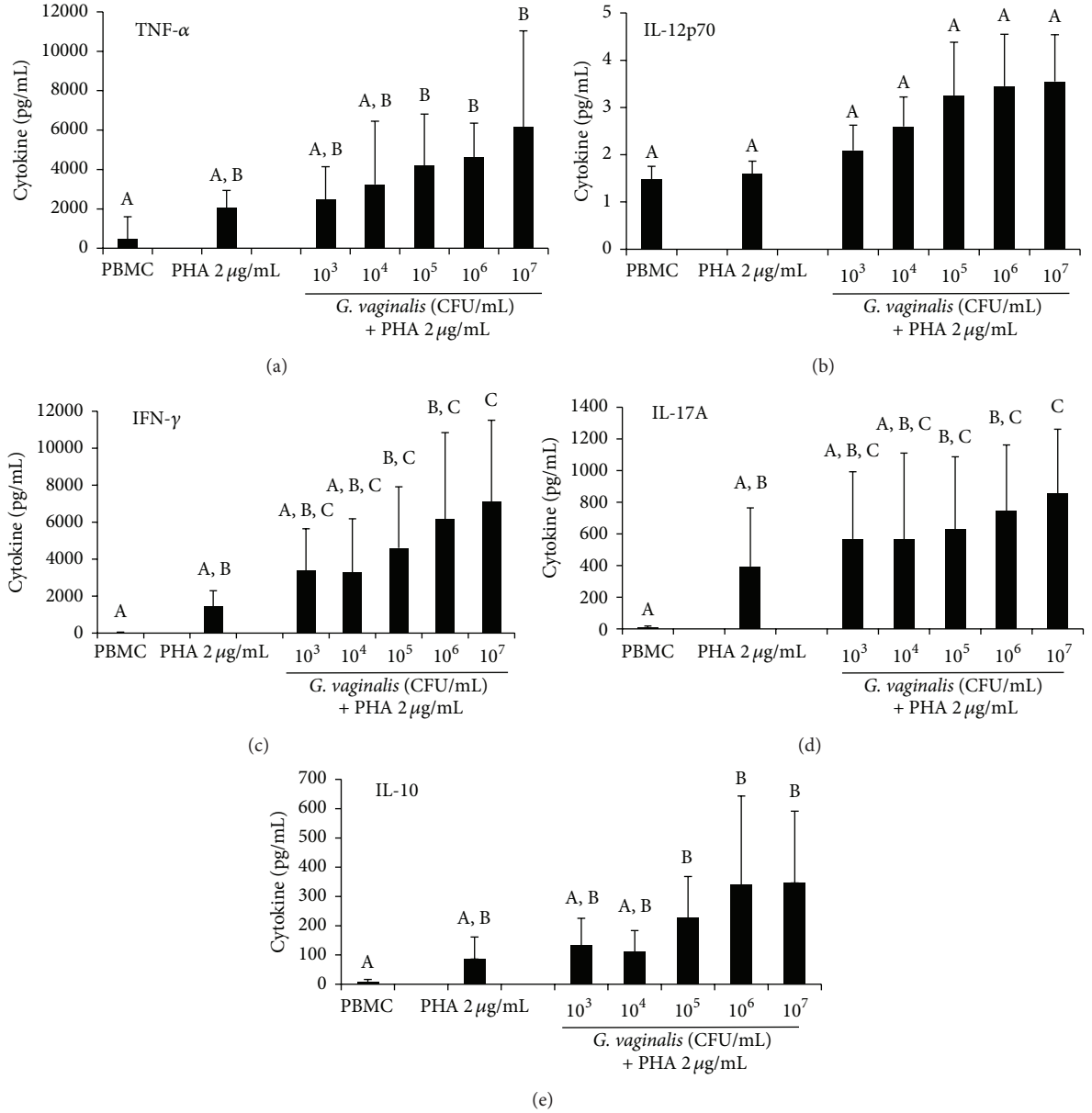


Figure 7: Modulation of PHA-induced cytokine secretion after exposure to a range of *G. vaginalis* concentrations. (a) TNF-α secretion ($n = 6$). (b) IL-12p70 secretion ($n = 8$). (c) IFN-γ secretion ($n = 7$). (d) IL-17a secretion ($n = 8$). (e) IL-10 secretion ($n = 8$). Compared to the PHA-stimulated control cells, a significant and dose-dependent increase in cytokine secretion was observed for IL-17a and IFN-γ with the *G. vaginalis*-treated cells. Other cytokines showed no significant increase in secretion. Data are means (+ SD). PBMC: PBMCs cultured for 72 h without any effector; PHA control: PBMCs only exposed to PHA (2 μg/mL) for 72 h; *G. vaginalis*: PBMCs exposed for 72 h to PHA (2 μg/mL) in the presence of 10^3 to 10^7 CFU/mL of *G. vaginalis*. Friedman's test with Nemenyi's comparison ($p < 0.05$). A, B, and C: means with different superscript letters are significantly different from each other.

show the potential immunological effects of the bacteria of the vaginal flora and suggest the existence of a mechanism whereby BV and its associated changes in flora composition could affect host vaginal immunity. Finally, these results lend weight to the putative role of *G. vaginalis* in the pathophysiology of BV and open up broader prospects, in particular for the understanding of the contribution of local immunological alterations to the increased risk of STIs in women with BV.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors thank the CICS of Clermont-Ferrand and are very grateful to Christelle Blavignac, Claire Szczepaniak, and

Lorraine Novais Gameiro for their generous cooperation. This work was partially supported by a grant of the regional council of Auvergne.

References

[1] J. E. Allsworth and J. F. Peipert, "Prevalence of bacterial vaginosis: 2001–2004 National Health and Nutrition Examination Survey data," *Obstetrics and Gynecology*, vol. 109, no. 1, pp. 114–120, 2007.

[2] H. Leitich, B. Bodner-Adler, M. Brunbauer, A. Kaider, C. Egarter, and P. Husslein, "Bacterial vaginosis as a risk factor for preterm delivery: a meta-analysis," *American Journal of Obstetrics and Gynecology*, vol. 189, no. 1, pp. 139–147, 2003.

[3] S. L. Hillier, R. P. Nugent, D. A. Eschenbach et al., "Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant," *The New England Journal of Medicine*, vol. 333, no. 26, pp. 1737–1742, 1995.

[4] J. Atashili, C. Poole, P. M. Ndumbe, A. A. Adimora, and J. S. Smith, "Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies," *AIDS*, vol. 22, no. 12, pp. 1493–1501, 2008.

[5] N. Sewankambo, R. H. Gray, M. J. Wawer et al., "HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis," *The Lancet*, vol. 350, no. 9077, pp. 546–550, 1997.

[6] E. H. Koumans, L. E. Markowitz, and V. Hogan, "Indications for therapy and treatment recommendations for bacterial vaginosis in nonpregnant and pregnant women: a synthesis of data," *Clinical Infectious Diseases*, vol. 35, no. 2, pp. S152–S172, 2002.

[7] M. A. D. Antonio, S. E. Hawes, and S. L. Hillier, "The identification of vaginal *Lactobacillus* species and the demographic and microbiologic characteristics of women colonized by these species," *The Journal of Infectious Diseases*, vol. 180, no. 6, pp. 1950–1956, 1999.

[8] D. N. Fredricks, T. L. Fiedler, and J. M. Marrazzo, "Molecular identification of bacteria associated with bacterial vaginosis," *The New England Journal of Medicine*, vol. 353, no. 18, pp. 1899–1911, 2005.

[9] U. Forsum, E. Holst, P. G. Larsson, A. Vasquez, T. Jakobsson, and I. Mattsby-Baltzer, "Bacterial vaginosis—a microbiological and immunological enigma," *APMIS*, vol. 113, no. 2, pp. 81–90, 2005.

[10] J. R. Schwebke, "New concepts in the etiology of bacterial vaginosis," *Current Infectious Disease Reports*, vol. 11, no. 2, pp. 143–147, 2009.

[11] H. L. Gardner and C. D. Dukes, "New etiologic agent in nonspecific bacterial vaginitis," *Science*, vol. 120, no. 3125, p. 853, 1954.

[12] W. E. Josey and J. R. Schwebke, "The polymicrobial hypothesis of bacterial vaginosis causation: a reassessment," *International Journal of STD and AIDS*, vol. 19, no. 3, pp. 152–154, 2008.

[13] P. A. Mårdh, E. Holst, and B. R. Møller, "The grivet monkey as a model for study of vaginitis. Challenge with anaerobic curved rods and Gardnerella vaginalis," *Scandinavian Journal of Urology and Nephrology Supplement*, vol. 86, pp. 201–205, 1984.

[14] J. D. Sobel, "Vaginitis," *The New England Journal of Medicine*, vol. 337, no. 26, pp. 1896–1903, 1997.

[15] A. Swidsinski, W. Mendling, V. Loening-Baucke et al., "An adherent *Gardnerella vaginalis* biofilm persists on the vaginal epithelium after standard therapy with oral metronidazole," *American Journal of Obstetrics and Gynecology*, vol. 198, no. 1, pp. 97.e1–97.e6, 2008.

[16] N. Cools, P. Ponsaerts, V. F. I. Van Tendeloo, and Z. N. Berneman, "Balancing between immunity and tolerance: an interplay between dendritic cells, regulatory T cells, and effector T cells," *Journal of Leukocyte Biology*, vol. 82, no. 6, pp. 1365–1374, 2007.

[17] J. Sabatté, J. Maggini, K. Nahmod et al., "Interplay of pathogens, cytokines and other stress signals in the regulation of dendritic cell function," *Cytokine and Growth Factor Reviews*, vol. 18, no. 1-2, pp. 5–17, 2007.

[18] A. Mazzoni and D. M. Segal, "Controlling the Toll road to dendritic cell polarization," *Journal of Leukocyte Biology*, vol. 75, no. 5, pp. 721–730, 2004.

[19] J. Zhu and W. E. Paul, "Heterogeneity and plasticity of T helper cells," *Cell Research*, vol. 20, no. 1, pp. 4–12, 2010.

[20] T. Kaldensjö, P. Petersson, A. Tolf, G. Morgan, K. Broliden, and T. Hirbod, "Detection of intraepithelial and stromal langerin and CCR5 positive cells in the human endometrium: potential targets for HIV infection," *PLoS ONE*, vol. 6, no. 6, Article ID e21344, 2011.

[21] D. Duluc, J. Gannevat, E. Anguiano et al., "Functional diversity of human vaginal APC subsets in directing T-cell responses," *Mucosal Immunology*, vol. 6, no. 3, pp. 626–638, 2013.

[22] L. de Witte, A. Nabatov, and T. B. H. Geijtenbeek, "Distinct roles for DC-SIGN$^+$-dendritic cells and Langerhans cells in HIV-1 transmission," *Trends in Molecular Medicine*, vol. 14, no. 1, pp. 12–19, 2008.

[23] E. P. St John, J. Martinson, J. A. Simoes, A. L. Landay, and G. T. Spear, "Dendritic cell activation and maturation induced by mucosal fluid from women with bacterial vaginosis," *Clinical Immunology*, vol. 125, no. 1, pp. 95–102, 2007.

[24] E. P. St John, M. R. Zariffard, J. A. Martinson, J. A. Simoes, A. L. Landay, and G. T. Spear, "Effect of mucosal fluid from women with bacterial vaginosis on HIV trans-infection mediated by dendritic cells," *Virology*, vol. 385, no. 1, pp. 22–27, 2009.

[25] J. J. Schellenberg, C. M. Card, T. B. Ball et al., "Bacterial vaginosis, HIV serostatus and T-cell subset distribution in a cohort of East African commercial sex workers: retrospective analysis," *AIDS*, vol. 26, no. 3, pp. 387–393, 2012.

[26] G. Anton, J. Rid, I. Mylonas, K. Friese, and E.-R. Weissenbacher, "Evidence of a TH1-shift of local vaginal inflammatory response during bacterial vaginosis," *Infection*, vol. 36, no. 2, pp. 147–152, 2008.

[27] S. Cauci, S. Guaschino, D. De Aloysio et al., "Interrelationships of interleukin-8 with interleukin-1β and neutrophils in vaginal fluid of healthy and bacterial vaginosis positive women," *Molecular Human Reproduction*, vol. 9, no. 1, pp. 53–58, 2003.

[28] S. Cauci, J. F. Culhane, M. Di Santolo, and K. McCollum, "Among pregnant women with bacterial vaginosis, the hydrolytic enzymes sialidase and prolidase are positively associated with interleukin-1β," *American Journal of Obstetrics and Gynecology*, vol. 198, no. 1, pp. 132.e1–132.e7, 2008.

[29] C. Mitchell and J. Marrazzo, "Bacterial vaginosis and the cervicovaginal immune response," *American Journal of Reproductive Immunology*, vol. 71, no. 6, pp. 555–563, 2014.

[30] C.-Y. Li, H.-C. Lin, C.-H. Lai, J. J.-Y. Lu, S.-F. Wu, and S.-H. Fang, "Immunomodulatory effects of *Lactobacillus* and *Bifidobacterium* on both murine and human mitogen-activated T cells," *International Archives of Allergy and Immunology*, vol. 156, no. 2, pp. 128–136, 2011.

[31] M. Rescigno, M. Urbano, B. Valzasina et al., "Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria," *Nature Immunology*, vol. 2, no. 4, pp. 361–367, 2001.

[32] D. M. LeBlanc, M. M. Barousse, and P. L. Fidel Jr., "Role for dendritic cells in immunoregulation during experimental vaginal candidiasis," *Infection and Immunity*, vol. 74, no. 6, pp. 3213–3221, 2006.

[33] A. Swidsinski, W. Mendling, V. Loening-Baucke et al., "Adherent biofilms in bacterial vaginosis," *Obstetrics & Gynecology*, vol. 106, no. 5, part 1, pp. 1013–1023, 2005.

[34] J. W. Costerton, P. S. Stewart, and E. P. Greenberg, "Bacterial biofilms: a common cause of persistent infections," *Science*, vol. 284, no. 5418, pp. 1318–1322, 1999.

[35] S. Manicassamy and B. Pulendran, "Dendritic cell control of tolerogenic responses," *Immunological Reviews*, vol. 241, no. 1, pp. 206–227, 2011.

[36] M. B. Lutz and G. Schuler, "Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity?" *Trends in Immunology*, vol. 23, no. 9, pp. 445–449, 2002.

[37] A. N. Masfari, B. I. Duerden, and G. R. Kinghorn, "Quantitative studies of vaginal bacteria," *Genitourinary Medicine*, vol. 62, no. 4, pp. 256–263, 1986.

[38] C. A. Spiegel, R. Amsel, D. Eschenbach, F. Schoenknecht, and K. K. Holmes, "Anaerobic bacteria in nonspecific vaginitis," *The New England Journal of Medicine*, vol. 303, no. 11, pp. 601–607, 1980.

[39] B. Evrard, D. Balestrino, A. Dosgilbert et al., "Roles of capsule and lipopolysaccharide O antigen in interactions of human monocyte-derived dendritic cells and *Klebsiella pneumoniae*," *Infection and Immunity*, vol. 78, no. 1, pp. 210–219, 2010.

[40] B. Evrard, S. Coudeyras, A. Dosgilbert et al., "Dose-dependent immunomodulation of human dendritic cells by the probiotic *Lactobacillus rhamnosus* Lcr35," *PLoS ONE*, vol. 6, no. 4, Article ID e18735, 2011.

[41] A. R. Goepfert, M. Varner, K. Ward et al., "Differences in inflammatory cytokine and Toll-like receptor genes and bacterial vaginosis in pregnancy," *American Journal of Obstetrics & Gynecology*, vol. 193, no. 4, pp. 1478–1485, 2005.

[42] M. R. Zariffard, R. M. Novak, N. Lurain, B. E. Sha, P. Graham, and G. T. Spear, "Induction of tumor necrosis factor-α secretion and toll-like receptor 2 and 4 mRNA expression by genital mucosal fluids from women with bacterial vaginosis," *Journal of Infectious Diseases*, vol. 191, no. 11, pp. 1913–1921, 2005.

[43] S. Cauci, "Vaginal immunity in bacterial vaginosis," *Current Infectious Disease Reports*, vol. 6, no. 6, pp. 450–456, 2004.

Hindawi Publishing Corporation
Journal of Immunology Research
Volume 2016, Article ID 5290604, 12 pages
http://dx.doi.org/10.1155/2016/5290604

Research Article

Group B *Streptococcus* Induces a Robust IFN-γ Response by CD4$^+$ T Cells in an *In Vitro* and *In Vivo* Model

Damian Clarke,[1] Corinne Letendre,[1] Marie-Pier Lecours,[1] Paul Lemire,[1] Tristan Galbas,[2] Jacques Thibodeau,[2] and Mariela Segura[1]

[1]*Laboratory of Immunology, Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, QC, Canada J2S 2M2*
[2]*Laboratory of Molecular Immunology, Department of Microbiology, Infectiology and Immunology, University of Montreal, Montreal, QC, Canada H3C 3J7*

Correspondence should be addressed to Mariela Segura; mariela.segura@umontreal.ca

Received 23 October 2015; Accepted 6 January 2016

Academic Editor: Daniela Rosa

Group B *Streptococcus* (GBS) serotype III causes life-threatening infections. Cytokines have emerged as important players for the control of disease, particularly IFN-γ. Although potential sources of this cytokine have been proposed, no specific cell line has ever been described as a leading contributor. In this study, CD4$^+$ T cell activation profiles in response to GBS were evaluated through *in vivo*, *ex vivo,* and *in vitro* approaches. Total splenocytes readily produce a type 1 proinflammatory response by releasing IFN-γ, TNF-α, and IL-6 and actively recruit T cells via chemokines like CXCL9, CXCL10, and CCL3. Responding CD4$^+$ T cells differentiate into Th1 cells producing large amounts of IFN-γ, TNF-α, and IL-2. *In vitro* studies using dendritic cell and CD4$^+$ T cell cocultures infected with wild-type GBS or a nonencapsulated mutant suggested that GBS capsular polysaccharide, one of the major bacterial virulence factors, differentially modulates surface expression of CD69 and IFN-γ production. Overall, CD4$^+$ T cells are important producers of IFN-γ and might thus influence the course of GBS infection through the expression balance of this cytokine.

1. Introduction

Group B *Streptococcus* (GBS) or *Streptococcus agalactiae* is the main cause of life-threatening infections in newborns worldwide [1, 2]. GBS also affects pregnant women, elders, and immunocompromised patients [3]. Type III GBS is frequently involved in neonatal infections and is the most common type in GBS meningitis [1, 2].

Cytokines are important for controlling GBS disease, although exaggerated responses might be dangerous [4, 5]. While IL-10, IL-12, and IL-18 are beneficial [6–9], TNF-α contributes to GBS-induced sepsis [7, 10]. IFN-γ appears promising for control of GBS disease; IL-12 and IL-18 exert therapeutic effects by stimulating immune cells to produce IFN-γ [6, 8, 9], IFN-γ production is impaired in neonates and this might partly explain their susceptibility to GBS infection [8, 11, 12], and IFN-γ inhibits GBS survival in human endothelial cells [13]. Although NK and NKT cells have been proposed to secrete IFN-γ in response to GBS [14, 15], no specific cell line has been clearly identified yet as a major source.

Activated CD4$^+$ T cells can differentiate into T helper (Th) cell types depending on the signals they receive. Th1 cells readily produce IFN-γ upon activation. GBS-infected dendritic cells (DCs) produce large amounts of proinflammatory cytokines like TNF-α, IL-6, and IL-12 [16] that could activate T cells. Furthermore, GBS-activated DCs release chemokines recruiting T cells, like CXCL9 and CXCL10 [16]. Although these evidences support IFN-γ production by T cells [17, 18], the participation of CD4$^+$ T cells during GBS-induced disease is unknown.

GBS possesses a thick sialylated polysaccharide capsule (CPS) [19]. It is known as the most important factor for GBS survival within the host and interferes with innate defense mechanisms [4, 20, 21]. Encapsulated GBS is highly internalized by DCs but survives better intracellularly than its nonencapsulated counterpart. Bacterial internalization and the presence of CPS are also related to modulation of several

cytokines and chemokines released by GBS-infected DCs [16, 22, 23].

It is hypothesized here that GBS drives $CD4^+$ T cells differentiation into IFN-γ-producing Th1 cells and that the CPS can modify this response. The role of $CD4^+$ T cells in the immune response against GBS type III was investigated using *in vivo*, *ex vivo*, and *in vitro* approaches in a mouse model. A nonencapsulated GBS mutant was included to dissect the role of this virulence factor in T cell activation.

2. Materials and Methods

2.1. Bacterial Strains. COH-1, a highly encapsulated type III GBS isolate extensively described in [16, 22, 24], and its isogenic nonencapsulated (Δ*cpsE*) mutant [16, 22] were used. GBS strains were cultivated as described previously [22].

2.2. Antibodies. Anti-mouse antibodies (BioLegend unless otherwise noted) used for FACS analysis were as follows: FITC-conjugated anti-CD3 (17A2) and anti-CD4 (GK1.5; BD Pharmingen); PE-conjugated anti-CD4 (GK1.5), anti-CD19 (6D5), anti-CD69 (H1.2F3; BD Pharmingen), anti-IFN-γ (XMG1.2; eBioscience), anti-TNF-α (MP6-XT22; eBioscience), and anti-IL-2 (JES6-5H4; eBioscience); PE-Cy7-conjugated anti-NK-1.1 (PK136) and anti-CD44 (IM7; BD Pharmingen); APC-conjugated anti-IFN-γ (XMG1.2), anti-TNF-α (MP6-XT22) and anti-IL-7Rα (A7R34), and BV421-conjugated anti-CD62L (MEL-14).

2.3. Mice and Experimental Infections. Five-week-old female C57BL/6 mice (Charles River Laboratories) were used for all experiments. The University of Montreal Animal Welfare Committee guidelines and policies were followed. On the day of the experiment, 0.5 mL of the bacterial suspension (10^6, 10^7, or 10^8 CFU) or sterile vehicle solution was administrated intraperitoneally (i.p.). Mortality and clinical signs were monitored [25]. Blood samples (5 μL) were collected at different times after infection. Bacteremia (number of CFU/mL) was determined by plating samples onto blood agar using an Automated Spiral Plater (Spiral Biotech).

2.4. Generation of Bone Marrow-Derived DCs and Isolation of Splenic $CD4^+$ T Cells. DCs were generated as described previously from naïve mice [16]. Cell purity was 86–90%$CD11c^{high}$ and $F4/80^{-/dim}$ cells by FACS analysis as reported previously [16]. For purification of untouched $CD4^+$ T cells, spleens (from either naïve or infected mice) were harvested, perfused with RPMI complete medium (Gibco), and pressed gently through a sterile fine wire mesh. After red blood cells lysis (eBioscience), total splenocytes were suspended in 2 mM EDTA-PBS solution and separated using Lympholyte-M density gradient (Cedarlane Lab.). Low-density cells at the interphase were purified by magnetic-activated cell sorting (MACS) negative selection (Miltenyi Biotec). The enriched $CD4^+$ T cells had >96% purity by FACS analysis using CD3 and CD4 antibodies (data not shown). For all experiments, cells were incubated at 37°C, 5% CO_2.

2.5. In Vivo Infection Model. For survival curves and selection of the infectious dose, mice ($n = 16$) were injected i.p. with 10^6, 10^7, or 10^8 CFU (strain COH-1) and clinical signs were monitored. Based on the obtained data (Figure 1(a)), mice were injected i.p. with 10^6 CFU. Surviving animals who displayed clinical signs were boosted with 10^6 CFU 2 weeks after initial infection. Bacteremia was monitored during 72 h after primary infection or at 24 h after boost. Spleens of animals with clinical signs and positive bacteremia were harvested 96 h after primary infection or 48 h after boost ($n = 2$ per group × 5 individual experiments). Five hours before spleen collection, mice were injected i.p. with 200 μg of Brefeldin A (eBioscience), a protein transport inhibitor. Control (mock-infected) animals were similarly treated. Brefeldin A was kept throughout the purification steps. The selected time points are based on pretrials analysis (data not shown). Purified $CD4^+$ T cells were analyzed for cytokine production by intracellular flow cytometry (IC-FACS). Total splenocytes were analyzed for memory surface markers by multiparametric FACS. Cells were gated on $CD3^+$ $CD4^+$ double-positive cells, followed by gating $CD44^{high}$ $CD62L^-$ (effector [memory] T cells) and $CD44^{high}$ $CD62L^+$ (central memory T cells). Analysis with a fifth surface marker, IL-7Rα^+, was used to further identify memory cells ($CD44^{high}$ IL-7Rα^+) within these two subsets [26, 27].

2.6. Ex Vivo Analysis of Total Splenocytes. Mice were injected i.p with 10^7 CFU (strain COH-1) ($n = 3$ per group × 3 individual experiments). Spleens were harvested 6 h after infection. Total splenocytes (5×10^6 cells/mL) were plated in complete medium without antibiotics and incubated for 48 and 72 h. After an initial 4 h incubation, the bacteriostatic agent chloramphenicol (12 μg/mL, Sigma-Aldrich) was added to control the bacterial load as reported previously [16]. Total splenocytes from control (mock-infected) animals were similarly treated. Concanavalin A (ConA, 0.1 μg/mL, Sigma-Aldrich) served as positive control. Supernatants were harvested at different time points for cytokine analysis. In selected experiments, Brefeldin A (3 μg/mL) was added for the last 5 h of incubation, and total splenocytes or $CD4^+$ T cells (MACS-isolated from the culture wells) were analyzed by IC-FACS after a total 48 h incubation. The culture conditions were selected based on pretrials (data not shown).

2.7. In Vitro DC-T Cell Coculture Model. DCs were plated in 48-well flat-bottom plates (10^5 cells/well; 1 h) prior to a 1 h infection with COH-1 or Δ*cpsE* strains (MOI:1). After a 1 h treatment with 100 μg/mL gentamycin and 5 μg/mL penicillin G (Sigma-Aldrich) to kill extracellular bacteria as described previously [16], DCs were washed. Freshly isolated $CD4^+$ T cells from naïve mice were added (5 : 1 T cell/DC ratio; 8 and 24 h). Cocultures incubated with medium alone or ConA (0.1 μg/mL) served as negative and positive controls, respectively. Cells were harvested for FACS analysis of surface marker expression. For T cell cytokine expression, after a 48 h incubation, plates were centrifuged and replenished with fresh medium containing 10 ng/mL of mouse rIL-2 (Miltenyi Biotec). After a 3-day resting period, T cells were harvested,

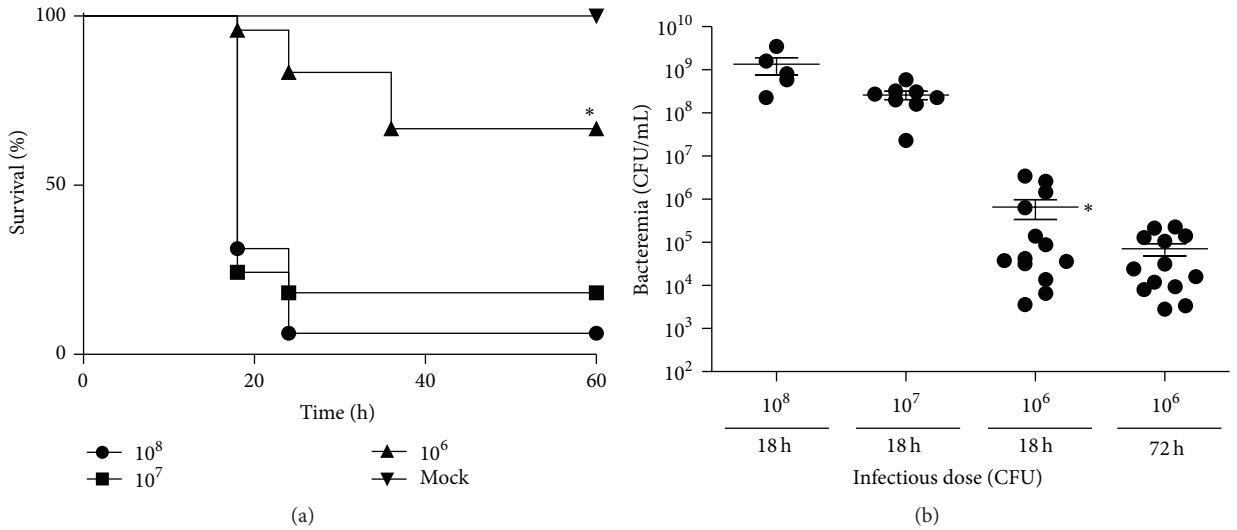


Figure 1: Survival curves and bacteremia levels of GBS-infected C57BL/6 mice. (a) Mice ($n = 16$) were injected intraperitoneally with different doses of wild-type GBS serotype III strain COH-1 and survival levels recorded. Mock-infected animals (injected with the vehicle solution) were used as controls. (b) Systemic bacteremia levels of infected mice were monitored at 18 h after infection (for mice infected with 10^6, 10^7, and 10^8 CFU) and at 72 h after infection (for mice infected with 10^6 CFU). Blood was drawn by tail puncture and serially diluted in PBS prior to plating on blood agar dishes. Individual colonies were counted and data expressed as CFU/mL of blood. $^*P < 0.05$, compared to higher infectious doses.

washed, and seeded into 96-well flat-bottom culture plates coated with 5 μg/mL of anti-mouse-CD3 mAb (BD Pharmingen) (10^5 cells/well; 48 h). Supernatants were harvested for ELISA testing. Single cell cultures (DCs or T cells alone) served as controls.

2.8. Cytokine and Chemokine Quantification by ELISA. Levels of IL-6, IL-10, IFN-γ, TNF-α, CCL3, CXCL9, and CXCL10 in cell culture supernatants were measured by sandwich ELISA using pair-matched antibodies (R&D Systems or eBioscience). Sample dilutions giving OD readings in the linear portion of a standard curve were used to quantify the levels of each cytokine. The results include at least three independent ELISA measurements.

2.9. FACS Analysis. For multiparametric IC-FACS, total splenocytes were treated with FcR-blocking reagent (FcγIII/II Rc Ab; BD Pharmingen) for 15 min on ice. Cells were stained for CD19, NK-1.1, CD3, and/or CD69 (30 min on ice), fixed, and permeabilized (eBioscience). After intracellular staining for IFN-γ or TNF-α (45 min, room temperature), FACS was performed using a FACSCanto II instrument (BD Biosciences). Fluorescence Minus One (FMO) control staining was performed for proper analysis and gating of target cells. For IC-FACS of MACS-purified $CD4^+$ T cells from *in vivo* or *ex vivo* experiments, cells were stained intracellularly for IFN-γ, TNF-α, and IL-2 as described above and analyzed with a FACSCalibur instrument (BD Biosciences). For analysis of the memory response by multiparametric FACS, total splenocytes were blocked and surface-stained for CD3, CD4, CD44, CD62L, and IL-7Rα (45 min on ice). FACS was performed using a FACSCanto II instrument. Cells from *in vitro* cocultures were surface-stained for CD4 and CD69 (30 min on ice). FACS was performed using a Cell Lab Quanta™ SC MPL MultiPlate Loader instrument (Beckman Coulter).

2.10. Statistical Analysis. Survival curves of infected mice were generated using Kaplan-Meier plots and log-rank (Mantel-Cox) tests allowed comparison between groups. Bacteremia levels were compared using the Mann-Whitney test. Cytokine data (expressed as means ± SEM) and FACS data were analyzed for significance using Student's unpaired t-test. All analyses were performed using the Sigma Plot System (Systat Software). A $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Survival of GBS-Infected Mice Is Dose-Dependent. After 18 h, infection with 10^7 or 10^8 CFU of COH-1 strain resulted in 75% and 69% mortality ($P > 0.05$), respectively (Figure 1(a)). Mortality continued to increase until 24 h after infection to 82% and 94% ($P > 0.05$), respectively, and was maintained until 60 h after infection when the experiment was terminated. Mice infected with 10^6 CFU were significantly less prone to mortality than mice from the other groups. At 18 h after infection, only a 6% mortality rate was observed, which was significantly lower than in the other groups ($P < 0.05$). Mortality continued to increase at 24 and 36 h after infection yet remained significantly lower than in mice infected with higher doses ($P < 0.05$). Indeed, mice infected with 10^7 or 10^8 CFU manifested intense clinical signs as early as 8 h after

infection, while 10^6 CFU usually induced less severe signs starting 12 h after infection.

Bacteremia induced by COH-1 infection was consistent with survival curves (Figure 1(b)). Mice infected with 10^7 or 10^8 CFU showed high bacteremia at 18 h after infection and reached an average of 2.6×10^8 and 1.3×10^9 CFU/mL, respectively. In contrast, mice infected with 10^6 CFU showed significantly lower bacteremia and reached an average of 5.7×10^5 CFU/mL. High mortality rates impeded follow-up of bacteremia in mice infected with high doses. However, in mice infected with 10^6 CFU, bacteremia slowly decreased, reaching an average of 7.2×10^4 CFU/mL at 72 h after infection.

3.2. Splenocytes Produce Type-1 Proinflammatory Cytokines in Response to Encapsulated GBS Infection. Before investigating T cell activation, the splenic immunological environment was characterized. Total splenocytes from mice infected with COH-1 strain were incubated *ex vivo* for 48 and 72 h (Figure 2). High amounts of IFN-γ, TNF-α, and IL-6 were detected ($P < 0.05$), suggesting a type-1 proinflammatory response. IL-10 was also upregulated in infected spleens, suggesting a homeostatic role. Important chemokines for T cell recruitment were also detected: CXCL9, CXCL10, and CCL3 ($P < 0.05$). It is worth noting that CXCL9 and CXCL10 are mainly released in response to IFN-γ activation [28], thus in agreement with the observed high levels of IFN-γ produced by GBS-infected splenocytes. No significant differences were observed between 48 and 72 h cultures, except for CXCL9 where maximal production was delayed to 72 h of incubation.

3.3. Activated $CD4^+$ T Cells Contribute to IFN-γ Production during Encapsulated GBS Infection. With current understanding of the splenic environment, the contribution of activated T cells to cytokine production was investigated. A multiparametric IC-FACS analysis of IFN-γ production from *ex vivo* total splenocytes cultures was performed. $CD3^+$ T cells markedly contributed to the IFN-γ response in the spleen of infected mice (Figure 3(a); $P < 0.05$). NKT cells ($NK1.1^+$ $CD3^+$) produced very low levels of IFN-γ (data not shown). NK cells ($NK1.1^+$) were major contributors to IFN-γ production within the $CD3^-$ population (data not shown). As expected, B cells ($CD19^+$) did not produce significant levels of this cytokine (data not shown). Activated $CD3^+$ T cells also contributed to approximately half the production of TNF-α by splenic cells (Figure 3(c); $P < 0.05$). Compared to control mice, splenocytes from infected animals showed a significant increase in surface expression of the early activation marker CD69. High expression of CD69 was also observed within the $CD3^+$ population (Figure 3(b); $P < 0.05$).

$CD4^+$ T cells were isolated from *ex vivo* total splenocyte cultures and analyzed by IC-FACS to specifically evaluate their role (Figure 4). Activated $CD4^+$ T cells contributed to the production of IFN-γ and TNF-α. Low levels of intracellular IL-2 were also observed (Figure 4). *In vivo* experiments confirmed these results; $CD4^+$ T cells directly isolated from the spleen of infected mice 96 h after primary infection showed that they contribute to the production of IFN-γ and TNF-α. Intracellular levels of IL-2 were hardly detected during a primary infection (Figure 5, black histograms). $CD4^+$ T cells isolated 48 h after boost displayed an enhanced contribution to IFN-γ, TNF-α, and IL-2 production (Figure 5, dark grey histograms). This is consistent with the generation of memory $CD4^+$ T cells ($CD44^{high}$ IL-$7R\alpha^+$) observed at that time with the increase in IL-$7R\alpha^+$ cells in the central memory subset ($CD44^{high}$ $CD62L^+$) (Figures 6(a) and 6(b), red population and histograms). The decrease in IL-$7R\alpha^+$ cells in the effector (memory) subset ($CD44^{high}$ $CD62L^-$) likely reflects cellular migration from the spleen to peripheral tissues (Figures 6(a) and 6(b), blue population and histograms).

3.4. The CPS of GBS Modulates Cytokine Release by $CD4^+$ T Cells. As GBS is a well-encapsulated bacterium, the impact of CPS on $CD4^+$ T cell activation was evaluated by comparing COH-1 with its nonencapsulated mutant, Δ*cpsE*, in an *in vitro* DC-T cell coculture system. Since nonencapsulated GBS mutants are rapidly cleared from circulation [20], *in vivo* comparison was impossible. Coculture supernatants were tested by ELISA for $CD4^+$ T cell-derived cytokines. No significant cytokine production was observed in single cell cultures (DCs or T cells alone) that served as controls (data not shown). COH-1-activated cocultures showed extremely high levels of IFN-γ (~45000 pg/mL) and significant levels of TNF-α (~1500 pg/mL). Δ*cpsE*-activated cocultures showed a significant reduction in IFN-γ production (Figure 7; $P = 0.012$), along with reduced TNF-α production, although this difference was not statistically significant ($P = 0.053$). Overall, these results suggest that nonencapsulated GBS-pulsed DCs induce reduced cytokine production by $CD4^+$ T cells compared to encapsulated GBS-pulsed DCs.

3.5. The CPS of GBS Affects Surface Expression of CD69 on Activated $CD4^+$ T Cells. In addition to cytokine production, expression of surface molecules is essential for proper T cell activation. The effect of CPS on CD69 expression on activated $CD4^+$ T cells was investigated. In COH-1-activated cocultures, CD69 expression on $CD4^+$ T cells was significantly lower than in Δ*cpsE*-activated cocultures after an 8 h incubation (Figure 8; $P < 0.01$). CD69 expression remained lower in COH-1-activated cocultures at 24 h ($P < 0.05$), although the difference and levels of expression were less pronounced. After 48 h, no significant differences in CD69 expression were observed between strains (data not shown).

4. Discussion

Although interactions between GBS and innate immune cells are increasingly documented, activation profiles of adaptive immune cells have never been investigated. This is the first study evaluating $CD4^+$ T cells contribution to GBS immune response using *in vivo*, *ex vivo*, and *in vitro* analysis.

While cytokines contribute to host defense development, they can also exacerbate GBS-induced pathologies. Initial *ex vivo* analysis of cytokine production by total splenocytes

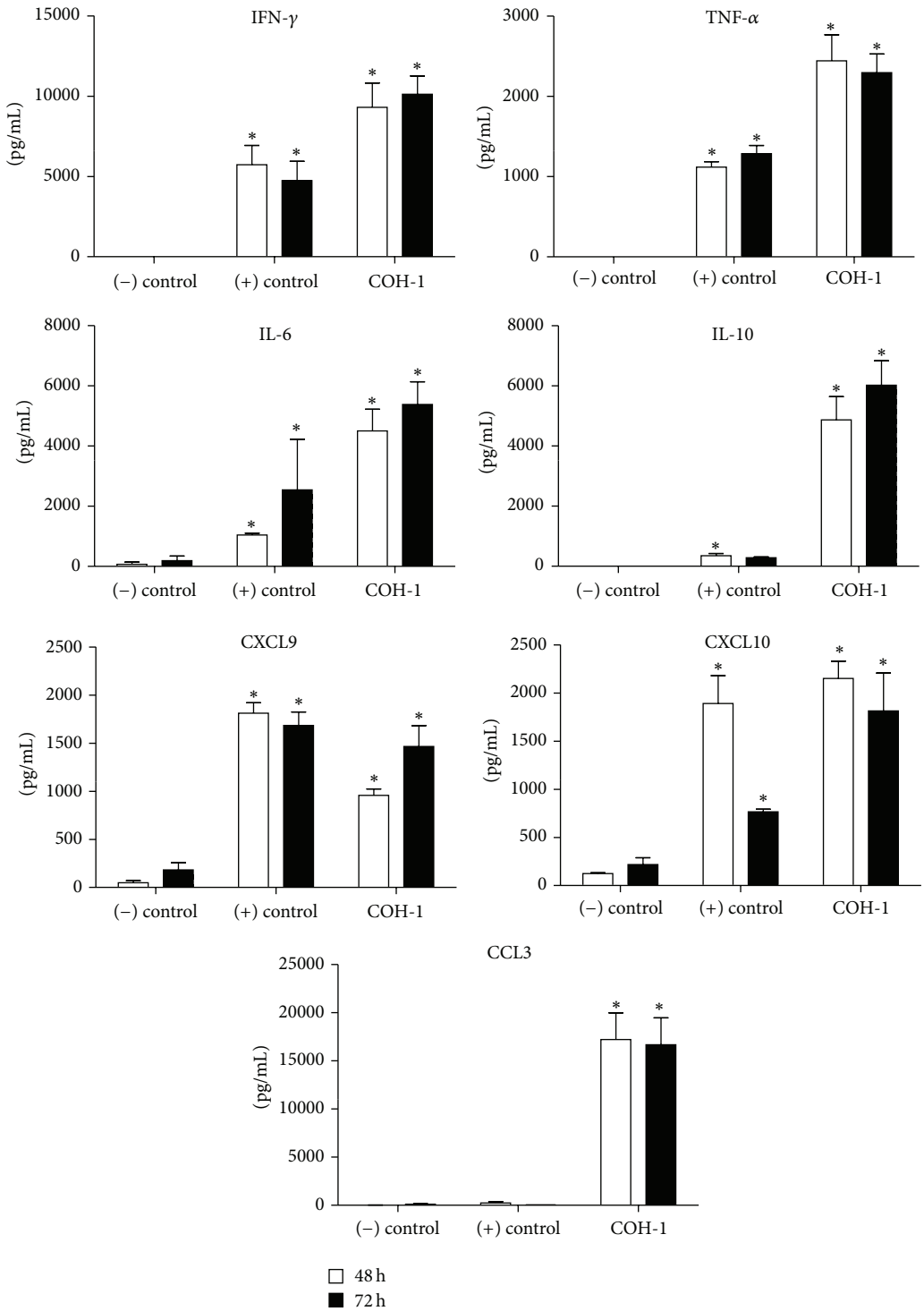


Figure 2: *Ex vivo* cytokine and chemokine production profile by total splenocytes. C57BL/6 mice were injected intraperitoneally with a dose of 10^7 CFU of wild-type GBS serotype III strain COH-1 ($n = 3$ per group $\times$ 3 individual experimental infections). Spleens were harvested 6 h after infection and total splenocytes plated at 5×10^6 cells/well. After 4 h of incubation, the bacteriostatic agent chloramphenicol (12 μg/mL) was added to the culture to prevent cell toxicity. Cells were then incubated for 48 h and 72 h and supernatants were collected for cytokine analysis by ELISA. Nonstimulated cells from mock-infected animals served as negative (−) control for basal expression. Cells stimulated with Concanavalin A (0.1 μg/mL) were used as positive (+) control. Data are expressed as means ± SEM (in pg/mL) from 3 different experimental infections. $^*P < 0.05$ indicates statistically significant difference compared to (−) control cells.

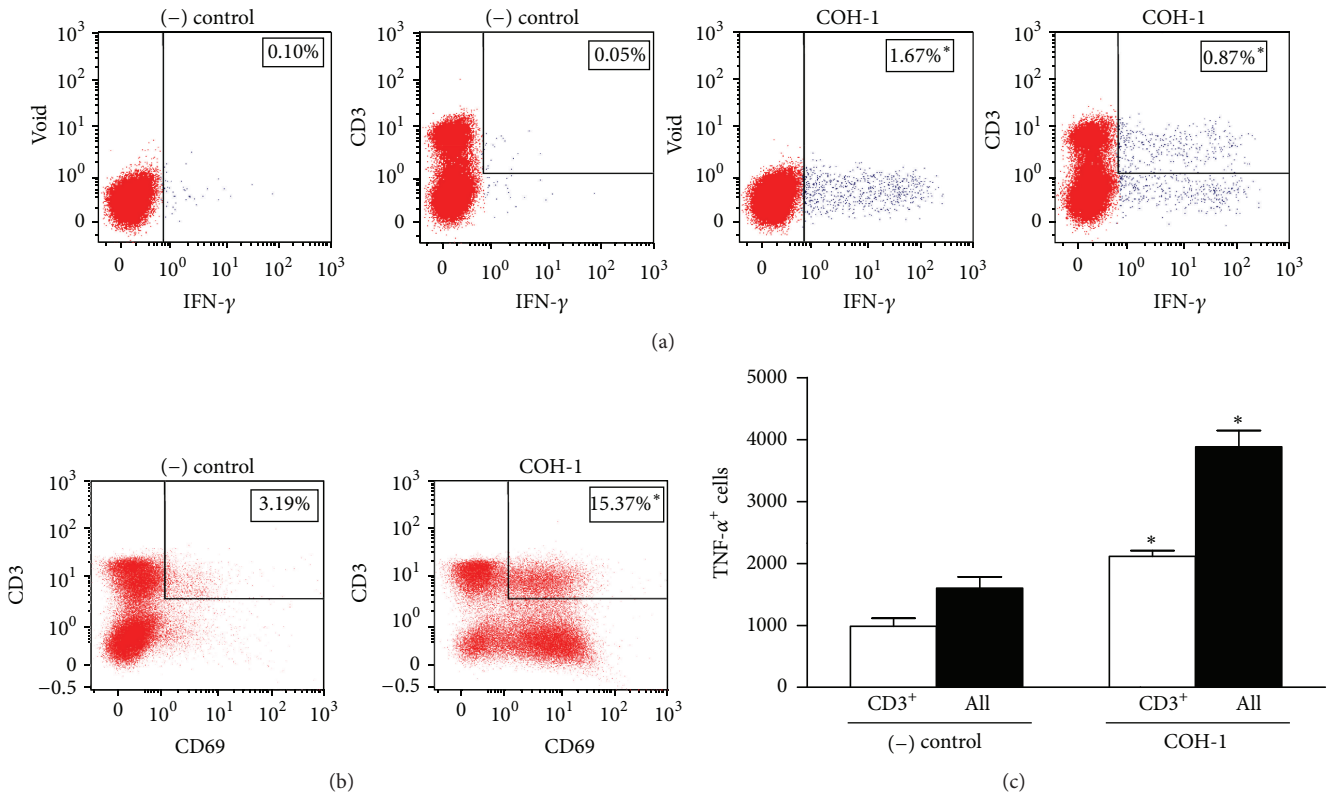


Figure 3: *Ex vivo* analyses of cellular sources of IFN-γ during GBS infection. C57BL/6 mice were injected intraperitoneally with a dose of 10^7 CFU of wild-type GBS serotype III strain COH-1 ($n = 3$ per group $\times$ 3 individual experimental infections). Spleens were harvested 6 h after infection and total splenocytes plated at 5×10^6 cells/well. After 4 h of incubation, the bacteriostatic agent chloramphenicol (12 μg/mL) was added to the culture to prevent cell toxicity. Nonstimulated cells from mock-infected animals served as negative (−) control for basal expression. Total splenocytes were incubated for 48 h with Brefeldin A (3 μg/mL) added during the last 5 h of incubation. Cells were harvested and intracellularly stained for IFN-γ (a) or surface-stained for CD69 (b) in combination with several surface markers for multiparametric FACS analysis. Representative data from 3 different experimental infections based on $CD3^+$ population or total splenic population (Void). (c) Number of TNF-α^+ cells within the $CD3^+$ population or total splenic population (All). Data are expressed as means ± SEM from 3 different experimental infections; $^*P < 0.05$ indicates statistically significant difference compared to (−) control cells. Fifty thousand gated events were acquired per sample and data analysis was performed using FACSDiva™ software.

from encapsulated GBS-infected mice revealed the presence of IFN-γ, TNF-α, IL-6, and IL-10. Production of IFN-γ, TNF-α, and IL-6 suggests a type-1 proinflammatory response being developed shortly after infection, while IL-10 production can be related to immune regulation. Interestingly, TNF-α and IL-6 have routinely been reported as mediators of GBS sepsis [7, 10]. This result might also highlight the homeostatic role of IL-10. Indeed, IL-10 was shown to reduce TNF-α and thus protect neonatal mice from developing GBS sepsis [7].

DCs, monocytes, and macrophages are known to secrete TNF-α, IL-6, and/or IL-10 when responding to GBS [16, 17, 29–31]. However, sources of IFN-γ remain poorly identified. Early works reporting IFN-γ production used GBS-infected total splenocytes or mixed mononuclear cells, without identifying the cellular source [8–10, 17]. The present study defined the role of T cells in IFN-γ production. *Ex vivo* and *in vivo* analysis showed that $CD4^+$ T cells are important producers of IFN-γ and TNF-α during GBS infection. Activated $CD4^+$ T cells also produce low, but still significant levels of IL-2, suggesting the development of a Th1 response. $CD4^+$ T cells produce the same pattern of cytokines more efficiently after a boost infection, likely thanks to the memory response [32]. An important contribution of NK cells to the IFN-γ response was also evidenced *in vivo*, in accordance with previous *in vitro* studies with splenocytes from severe combined immunodeficiency mice [15]. IFN-γ production by NKT cells was very limited during GBS infection, even at earlier time points (unpublished observations), although purified GBS glycolipids have been shown to activate NKT cells [14].

Early chemokine release by innate immune cells attracts T cells to the site of infection. *Ex vivo* analysis of chemokine production by total splenocytes suggested that T cells are actively recruited via CCL3, CXCL9, and CXCL10. Interestingly, CXCL9 and CXCL10 are two CXCR3 ligands, both induced by IFN-γ. CXCR3 is rapidly upregulated on naive T cells following activation and remains preferentially highly expressed on Th1 cells [28]. Different splenic cell types, like DCs, might produce these chemokines in response to GBS [16]. Although upregulation of *Cxcl10* gene expression was observed in mouse peritoneal macrophages [31], GBS was

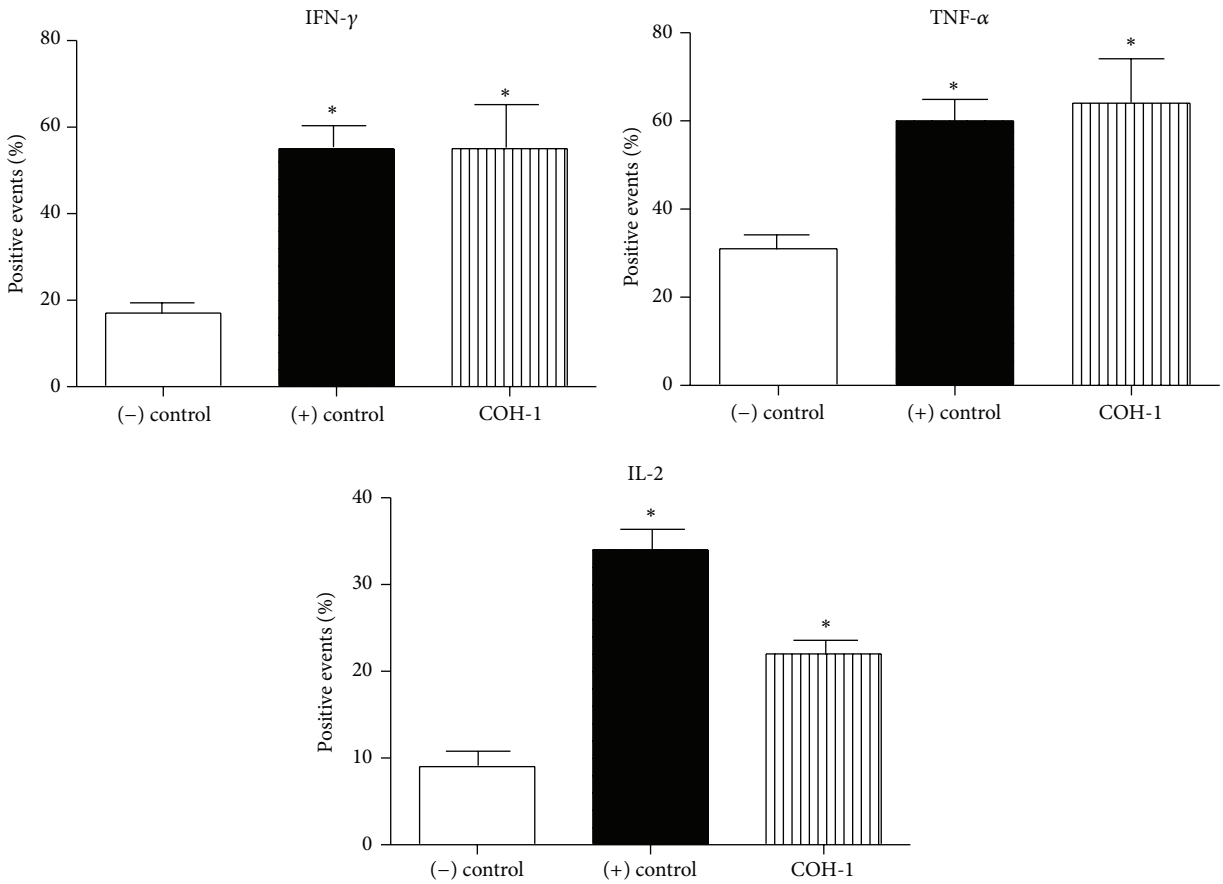


Figure 4: *Ex vivo* analyses of $CD4^+$ T cell contribution to cytokine production. C57BL/6 mice were injected intraperitoneally with a dose of 10^7 CFU of wild-type GBS serotype III strain COH-1 ($n = 3$ per group × 3 individual experimental infections). Spleens were harvested 6 h after infection and total splenocytes plated at 5×10^6 cells/well. After 4 h of incubation, the bacteriostatic agent chloramphenicol (12 μg/mL) was added to the culture to prevent cell toxicity. Nonstimulated cells from mock-infected animals served as negative (−) control for basal expression. Cells stimulated with Concanavalin A (0.1 μg/mL) were used as positive (+) control. Total splenocytes were incubated for 48 h. Brefeldin A (3 μg/mL) was added during the last 5 h of incubation and $CD4^+$ T cells were MACS-isolated from the culture, stained intracellularly for different cytokines, and analyzed by FACS. Data are expressed as means ± SEM (in % of positive cells) from 3 individual experimental infections. $^*P < 0.05$ indicates statistically significant difference compared to (−) control cells. Twenty thousand gated events were acquired per sample and data analysis was performed using CellQuest software. Histograms were drawn based on PE-control stain and were plotted on logarithmic scales.

unable to induce either CXCL10 or CXCL9 secretion by these cells [33]. Nevertheless, both macrophages and DCs seem to contribute to CCL3 production [31, 33, 34].

As GBS possesses a thick CPS, its most important virulence factor, the potential of CPS to modulate $CD4^+$ T cell activation was investigated. Similarly to *ex vivo* and *in vivo* results, DCs pulsed *in vitro* with encapsulated GBS induced the release of high levels of IFN-γ and TNF-α by $CD4^+$ T cells. The production of IFN-γ was significantly decreased with nonencapsulated GBS. Production of TNF-α was also reduced. It is surprising that the loss of capsule does not trigger an exaggerated response or increased IFN-γ production by T cells, as reported for other encapsulated pathogens [35–37]. However, studies on GBS-activated DCs have shown similar trends; encapsulated GBS induced similar or stronger cytokine production by infected DCs than nonencapsulated GBS-infected counterparts [16, 34]. The only exception was IL-10, where production was significantly higher in DCs infected with the nonencapsulated mutant [16]. Two interrelated hypotheses were suggested to explain these observations: (a) increased IL-10 production by DCs reduces the production of other cytokines; or (b) more efficient killing of the nonencapsulated mutant reduces cytokine production by DCs [16, 22]. Moreover, it was reported that the presence of CPS modulates the endocytic pathways used by DCs for GBS uptake [22]. Since the route of entry influences the repertoires of epitopes presented to $CD4^+$ T cells, the ensuing immune response might be affected [38]. Thus, in our DC-T cell coculture system, DC modulation by the nonencapsulated strain may lead to lower levels of IFN-γ production by $CD4^+$ T cells.

In contrast to cytokine production, the surface expression of CD69 was higher (early time points) or similar (late time

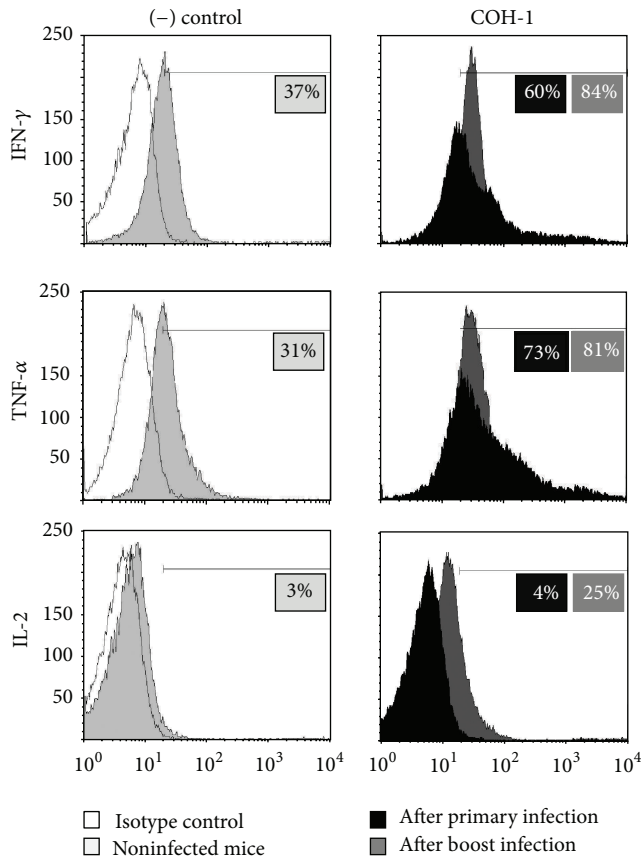


FIGURE 5: *In vivo* $CD4^+$ T cell contribution to cytokine production during primary and secondary GBS infections. C57BL/6 mice were injected intraperitoneally with a dose of 10^6 CFU of wild-type GBS serotype III strain COH-1. Surviving animals who had previously displayed clinical signs were boosted with a second dose of 10^6 CFU of GBS strain COH-1 two weeks after initial infection. Spleens of animals with clinical signs and positive bacteremia were harvested 96 h after primary infection or 48 h after boost infection ($n = 2$ per group $\times$ 5 individual experimental infections). Five hours prior to spleen collection, mice were injected with Brefeldin A (200 μg). (−) Control animals were similarly treated. Splenic $CD4^+$ T cells were MACS-purified, stained intracellularly for different cytokines, and analyzed by FACS. Representative data from 5 different experimental infections. Cytokine basal expression levels in (−) control animals were similar at 96 h after primary mock-infection and 48 h after secondary mock-infection. Representative histograms from the latter time point were selected for the figure. Twenty thousand gated events were acquired per sample and data analysis was performed using CellQuest software. Histograms were drawn based on PE-control stain and were plotted on logarithmic scales. It should be noted that isotype controls are the same in both groups, but only displayed on left panels to simplify the figure.

points) in $CD4^+$ T cells cocultured with nonencapsulated mutant-pulsed DCs compared to encapsulated GBS-infected cocultures. However, this could just be related to different kinetics of CD69 expression. In fact, attempting to explain modulation of CD69 expression on $CD4^+$ T cells is quite difficult, due to limited information on this marker. Indeed, characterization of its ligand has just started [39]. CD69 is known to be one of the earliest markers induced upon activation of T cells and acts as a signal-transmitting receptor for immunoregulatory events [40]. Of the few studies available on CD69 expression by T cells upon streptococcal infection, Harimaya et al. demonstrated a dose-dependent upregulation of CD69 on $CD3^+$ T cells from peripheral blood lymphocytes infected with *Streptococcus pneumoniae*. Yet, authors failed to correlate CD69 expression and IFN-γ production by these target cells [41]. More recently, in a *S. pneumoniae* mouse model of infection, $CD4^+$ T cells exhibited significant upregulation of CD69 in the spleen. As this response was MHC-II unrestricted, authors suggested that this increased CD69 expression on T cells might be due to secondary factors like cytokine release by other cells [42]. Likely, a polyclonal (indirect) activation of T cells in our system cannot be ruled out, although GBS failed to directly activate T cells without antigen-presenting cells (data not shown), similarly to that reported for *S. pneumoniae* [37, 42]. Finally, it has been suggested that CD69 plays an immunoregulatory role by preventing infection-induced immunopathology [43]. Enhanced expression of CD69 may result in reduced IFN-γ production by $CD4^+$ T cells [44].

5. Conclusion

Undoubtedly, IFN-γ production by $CD4^+$ T cells during GBS infection is crucial for host defense [8] but might also result

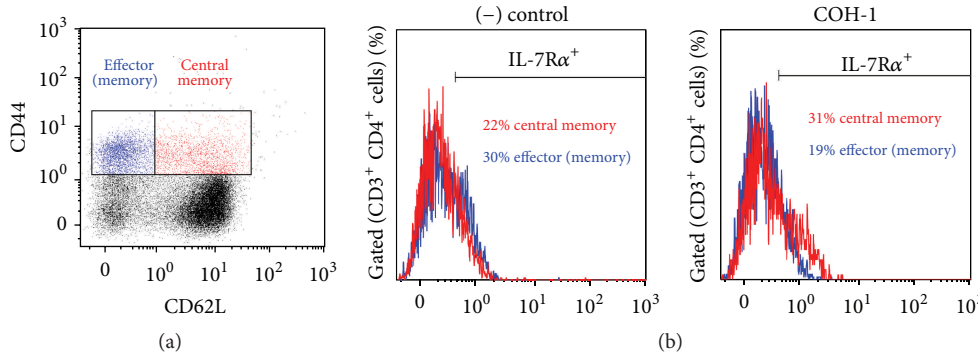


FIGURE 6: *In vivo* generation of memory $CD4^+$ T cells during GBS infection. C57BL/6 mice were injected intraperitoneally with a dose of 10^6 CFU of wild-type GBS serotype III strain COH-1. Surviving animals who had previously displayed clinical signs were boosted with a second dose of 10^6 CFU of GBS strain COH-1 two weeks after initial infection. Spleens of animals with clinical signs and positive bacteremia were harvested 48 h after boost infection. Total splenocytes were stained and analyzed by multiparametric FACS. (a) Cells were gated on $CD3^+$ $CD4^+$ double-positive cells, followed by gating $CD44^{high}$ $CD62L^-$ (effector [memory] T cells) and $CD44^{high}$ $CD62L^+$ (central memory T cells). A histogram from a representative control (mock-infected) mouse was selected for the figure. (b) A fifth surface marker, IL-7Rα^+, was used to further identify memory cells ($CD44^{high}$ IL-7Rα^+) within the $CD44^{high}$ $CD62L^-$ (effector [memory] T cells) and $CD44^{high}$ $CD62L^+$ (central memory T cells). IL-7Rα^+ cells reflect memory cells within these respective populations. Histograms from representative control (mock-infected) and infected mice were selected for the figure. Thirty thousand events gated on $CD3^+$ $CD4^+$ cells were acquired per sample and data analysis was performed using Kaluza® Flow Analysis software.

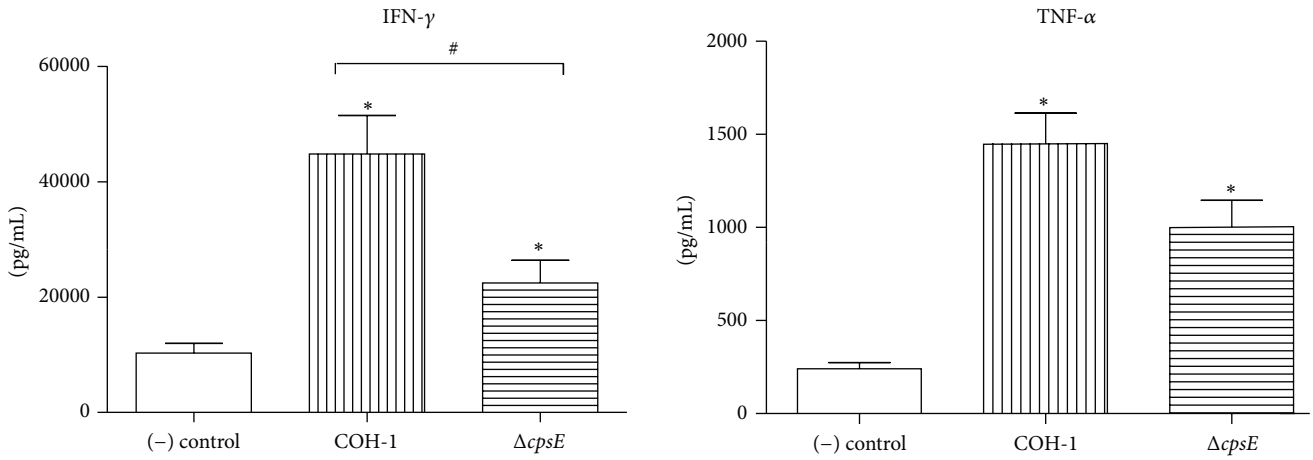


FIGURE 7: Role of bacterial capsular polysaccharide in the modulation of cytokine production by $CD4^+$ T cells. Dendritic cells (DCs) were infected with either wild-type GBS strain COH-1 or its nonencapsulated isogenic mutant Δ*cpsE* (MOI:1) for 1 h. Extracellular bacteria were killed by antibiotic treatment and cultures washed prior to addition of freshly isolated splenic $CD4^+$ T cells from naïve mice (T cell : DC ratio of 5 : 1). Cocultures were incubated for 48 h, resuspended in fresh medium containing 10 ng/mL of IL-2 for 72 h (resting period), and then transferred to anti-CD3 coated plates for 48 h. Supernatants were then collected and cytokines quantified by ELISA. Nonstimulated cocultures served as negative (−) controls for basal expression. Data are expressed as means ± SEM (in pg/mL) from 5 different experiments. $^*P < 0.05$ indicates statistically significant differences compared to (−) control. $^\#P < 0.05$ indicates statistically significant differences between cocultures infected with wild-type strain COH-1 and those infected with the nonencapsulated mutant Δ*cpsE*.

in disease pathology, as suggested in the mouse model of pneumococcal sepsis [42]. Although this study characterized for the first time IFN-γ production by $CD4^+$ T cells, a definitive understanding of all mechanisms regulating IFN-γ production during GBS infection requires further research. For instance, as the CPS confers a survival advantage to GBS [16, 22], persistence of GBS within antigen-presenting cells may affect their activation and thus the ensuing T cell immune response, including altered IFN-γ and CD69 expression balance early during infection.

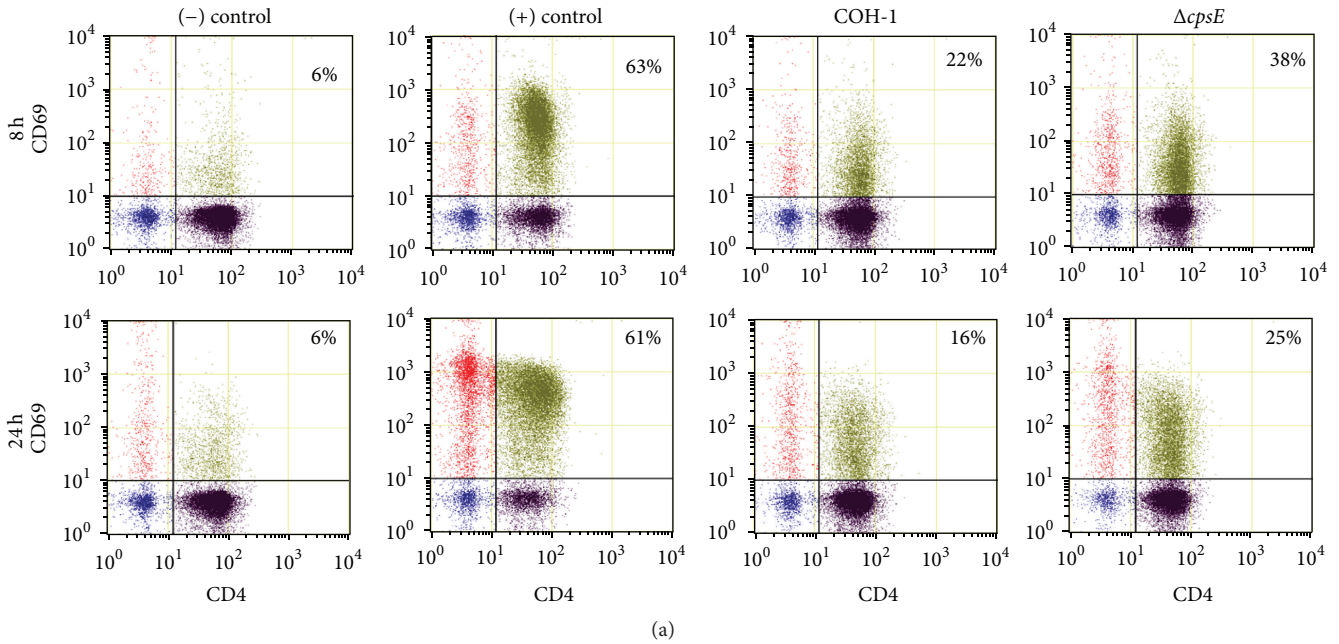


Figure 8: Role of bacterial capsular polysaccharide in the modulation of $CD4^+$ T cell surface expression of CD69. Dendritic cells (DCs) were infected with either wild-type GBS strain COH-1 or its nonencapsulated isogenic mutant Δ*cpsE* (MOI:1) for 1 h. Extracellular bacteria were killed by antibiotic treatment and cultures washed prior to addition of freshly isolated splenic $CD4^+$ T cells from naïve mice (T cell : DC ratio of 5 : 1). Cocultures were incubated for 8 h and 24 h, cells were harvested, and CD69 expression was analyzed by FACS. Cocultures incubated with medium alone or Concanavalin (0.1 μg/mL) served as negative (−) and positive controls (+), respectively. (a) Representative data from 3 different experiments. Twenty thousand gated events were acquired per sample and data analysis was performed using Cell Lab Quanta Collection/Analysis software. Quadrants were drawn based on FITC- and PE-control stains and were plotted on logarithmic scales. Numbers in the upper quadrants indicate the % of $CD4^+$ $CD69^+$ cells. (b) Data are expressed as means ± SEM from 3 different experimental infections; $^*P < 0.05$ indicates statistically significant differences between cocultures infected with wild-type strain COH-1 and those infected with the nonencapsulated mutant Δ*cpsE*.

Conflict of Interests

The authors declare that they have no conflict of interests in the research.

Authors' Contribution

Damian Clarke and Corinne Letendre contributed equally to this work.

Acknowledgments

This work was supported by Natural Sciences and Engineering Research Council of Canada (NSERC no. 342150-2013 to Mariela Segura) and by Canadian Institutes of Health Research (CIHR) and Canada Foundation for Innovation (CFI) through grants to Jacques Thibodeau (CIHR no. 93592). Corinne Letendre is the recipient of a M.S. award granted by NSERC. Marie-Pier Lecours and Paul Lemire are the recipients of Ph.D. awards granted by NSERC and by the Fonds de recherche du Québec-Nature et technologies, respectively.

References

[1] P. Melin, "Neonatal group B streptococcal disease: from pathogenesis to preventive strategies," *Clinical Microbiology and Infection*, vol. 17, no. 9, pp. 1294–1303, 2011.

[2] J. R. Verani and S. J. Schrag, "Group B streptococcal disease in infants: progress in prevention and continued challenges," *Clinics in Perinatology*, vol. 37, no. 2, pp. 375–392, 2010.

[3] M. S. Edwards and C. J. Baker, "Group B streptococcal infections in elderly adults," *Clinical Infectious Diseases*, vol. 41, no. 6, pp. 839–847, 2005.

[4] H. C. Maisey, K. S. Doran, and V. Nizet, "Recent advances in understanding the molecular basis of group B *Streptococcus* virulence," *Expert Reviews in Molecular Medicine*, vol. 10, article e27, 2008.

[5] J. Wennekamp and P. Henneke, "Induction and termination of inflammatory signaling in group B streptococcal sepsis," *Immunological Reviews*, vol. 225, no. 1, pp. 114–127, 2008.

[6] V. Cusumano, A. Midiri, V. V. Cusumano et al., "Interleukin-18 is an essential element in host resistance to experimental group B streptococcal disease in neonates," *Infection and Immunity*, vol. 72, no. 1, pp. 295–300, 2004.

[7] V. Cusumano, F. Genovese, G. Mancuso, M. Carbone, M. T. Fera, and G. Teti, "Interleukin-10 protects neonatal mice from lethal group B streptococcal infection," *Infection and Immunity*, vol. 64, no. 7, pp. 2850–2852, 1996.

[8] V. Cusumano, G. Mancuso, F. Genovese et al., "Role of gamma interferon in a neonatal mouse model of group B streptococcal disease," *Infection and Immunity*, vol. 64, no. 8, pp. 2941–2944, 1996.

[9] G. Mancuso, V. Cusumano, F. Genovese, M. Gambuzza, C. Beninati, and G. Teti, "Role of interleukin 12 in experimental neonatal sepsis caused by group B streptococci," *Infection and Immunity*, vol. 65, no. 9, pp. 3731–3735, 1997.

[10] G. Teti, G. Mancuso, and F. Tomasello, "Cytokine appearance and effects of anti-tumor necrosis factor alpha antibodies in a neonatal rat model of group B streptococcal infection," *Infection and Immunity*, vol. 61, no. 1, pp. 227–235, 1993.

[11] D. B. Lewis, A. Larsen, and C. B. Wilson, "Reduced interferon-gamma mRNA levels in human neonates. Evidence for an intrinsic T cell deficiency independent of other genes involved in T cell activation," *The Journal of Experimental Medicine*, vol. 163, no. 4, pp. 1018–1023, 1986.

[12] C. B. Wilson, "Immunologic basis for increased susceptibility of the neonate to infection," *The Journal of Pediatrics*, vol. 108, no. 1, pp. 1–12, 1986.

[13] V. D. O. F. Lione, M. H. B. dos Santos, J. S. S. de Oliveira, A. L. Mattos-Guaraldi, and P. E. Nagao, "Interferon-γ inhibits group B *Streptococcus* survival within human endothelial cells," *Memorias do Instituto Oswaldo Cruz*, vol. 109, no. 7, pp. 940–943, 2014.

[14] Y. Kinjo, P. Illarionov, J. L. Vela et al., "Invariant natural killer T cells recognize glycolipids from pathogenic Gram-positive bacteria," *Nature Immunology*, vol. 12, no. 10, pp. 966–974, 2011.

[15] C. A. Derrico and K. J. Goodrum, "Interleukin-12 and tumor necrosis factor alpha mediate innate production of gamma interferon by group B *Streptococcus*-treated splenocytes of severe combined immunodeficiency mice," *Infection and Immunity*, vol. 64, no. 4, pp. 1314–1320, 1996.

[16] P. Lemire, M. Houde, M.-P. Lecours, N. Fittipaldi, and M. Segura, "Role of capsular polysaccharide in Group B *Streptococcus* interactions with dendritic cells," *Microbes and Infection*, vol. 14, no. 12, pp. 1064–1076, 2012.

[17] D. J. Kwak, N. H. Augustine, W. G. Borges, J. L. Joyner, W. F. Green, and H. R. Hill, "Intracellular and extracellular cytokine production by human mixed mononuclear cells in response to group B streptococci," *Infection and Immunity*, vol. 68, no. 1, pp. 320–327, 2000.

[18] T. R. La Pine, J. L. Joyner, N. H. Augustine, S. D. Kwak, and H. R. Hill, "Defective production of IL-18 and IL-12 by cord blood mononuclear cells influences the T helper-1 interferon gamma response to group B streptococci," *Pediatric Research*, vol. 54, no. 2, pp. 276–281, 2003.

[19] M. J. Cieslewicz, D. Chaffin, G. Glusman et al., "Structural and genetic diversity of group B *Streptococcus* capsular polysaccharides," *Infection and Immunity*, vol. 73, no. 5, pp. 3096–3103, 2005.

[20] K. S. Doran and V. Nizet, "Molecular pathogenesis of neonatal group B streptococcal infection: no longer in its infancy," *Molecular Microbiology*, vol. 54, no. 1, pp. 23–31, 2004.

[21] A. F. Carlin, S. Uchiyama, Y.-C. Chang, A. L. Lewis, V. Nizet, and A. Varki, "Molecular mimicry of host sialylated glycans allows a bacterial pathogen to engage neutrophil Siglec-9 and dampen the innate immune response," *Blood*, vol. 113, no. 14, pp. 3333–3336, 2009.

[22] P. Lemire, M. Houde, and M. Segura, "Encapsulated group B *Streptococcus* modulates dendritic cell functions via lipid rafts and clathrin-mediated endocytosis," *Cellular Microbiology*, vol. 14, no. 11, pp. 1707–1719, 2012.

[23] A. Costa, R. Gupta, G. Signorino et al., "Activation of the NLRP3 inflammasome by group B *Streptococci*," *The Journal of Immunology*, vol. 188, no. 4, pp. 1953–1960, 2012.

[24] G. Mancuso, M. Gambuzza, A. Midiri et al., "Bacterial recognition by TLR7 in the lysosomes of conventional dendritic cells," *Nature Immunology*, vol. 10, no. 6, pp. 587–594, 2009.

[25] P. Lemire, C. Calzas, and M. Segura, "The NOD2 receptor does not play a major role in the pathogenesis of Group B *Streptococcus* in mice," *Microbial Pathogenesis*, vol. 65, pp. 41–47, 2013.

[26] R. Stephens and J. Langhorne, "Effector memory Th1 CD4 T cells are maintained in a mouse model of chronic malaria," *PLoS Pathogens*, vol. 6, no. 11, Article ID e1001208, 2010.

[27] M. N. Khan and M. E. Pichichero, "CD4 T cell memory and antibody responses directed against the pneumococcal histidine triad proteins PhtD and PhtE following nasopharyngeal colonization and immunization and their role in protection against pneumococcal colonization in mice," *Infection and Immunity*, vol. 81, no. 10, pp. 3781–3792, 2013.

[28] J. R. Groom and A. D. Luster, "CXCR3 in T cell function," *Experimental Cell Research*, vol. 317, no. 5, pp. 620–631, 2011.

[29] M. Bebien, M. E. Hensler, S. Davanture et al., "The pore-forming toxin β hemolysin/cytolysin triggers p38 MAPK-dependent IL-10 production in macrophages and inhibits innate immunity," *PLoS Pathogens*, vol. 8, no. 7, Article ID e1002812, 2012.

[30] M. A. De Francesco, F. Gargiulo, R. Negrini, M. Gelmi, and N. Manca, "Different sequence strains of *Streptococcus agalactiae* elicit various levels of cytokine production," *Immunological Investigations*, vol. 37, no. 8, pp. 741–751, 2008.

[31] D. W. Draper, H. N. Bethea, and Y.-W. He, "Toll-like receptor 2-dependent and -independent activation of macrophages by group B streptococci," *Immunology Letters*, vol. 102, no. 2, pp. 202–214, 2006.

[32] Q. Zhang, L. Bagrade, E. Clarke, J. C. Paton, D. A. Nunez, and A. Finn, "Bacterial lipoproteins differentially regulate human primary and memory CD4+ T and B cell responses to pneumococcal protein antigens through toll-like receptor 2," *Journal of Infectious Diseases*, vol. 201, no. 11, pp. 1753–1763, 2010.

[33] H. Fan, D. L. Williams, B. Zingarelli et al., "Differential regulation of lipopolysaccharide and Gram-positive bacteria induced cytokine and chemokine production in macrophages by $G\alpha_i$ proteins," *Immunology*, vol. 122, no. 1, pp. 116–123, 2007.

[34] P. Lemire, D. Roy, N. Fittipaldi et al., "Implication of TLR- but not of NOD2-signaling pathways in dendritic cell activation by group B *Streptococcus* serotypes III and V," *PLoS ONE*, vol. 9, no. 12, Article ID e113940, 2014.

[35] M.-P. Lecours, M. Gottschalk, M. Houde, P. Lemire, N. Fittipaldi, and M. Segura, "Critical role for *Streptococcus suis* cell wall modifications and suilysin in resistance to complement-dependent killing by dendritic cells," *Journal of Infectious Diseases*, vol. 204, no. 6, pp. 919–929, 2011.

[36] A. Kolb-Mäurer, A. Unkmeir, U. Kämmerer et al., "Interaction of *Neisseria meningitidis* with human dendritic cells," *Infection and Immunity*, vol. 69, no. 11, pp. 6912–6922, 2001.

[37] M. Olliver, J. Hiew, P. Mellroth, B. Henriques-Normark, and P. Bergman, "Human monocytes promote Th1 and Th17 responses to *Streptococcus pneumoniae*," *Infection and Immunity*, vol. 79, no. 10, pp. 4210–4217, 2011.

[38] A. von Delwig, E. Bailey, D. M. Gibbs, and J. H. Robinson, "The route of bacterial uptake by macrophages influences the repertoire of epitopes presented to CD4 T cells," *European Journal of Immunology*, vol. 32, no. 12, pp. 3714–3719, 2002.

[39] C. R. Lin, T. W. Wei, H. Y. Tsai, Y. T. Wu, P. Y. Wu, and S. T. Chen, "Glycosylation-dependent interaction between CD69 and S100A8/S100A9 complex is required for regulatory T-cell differentiation," *The FASEB Journal*, vol. 29, no. 12, pp. 5006–5017, 2015.

[40] S. F. Ziegler, F. Ramsdell, and M. R. Alderson, "The activation antigen CD69," *STEM CELLS*, vol. 12, no. 5, pp. 456–465, 1994.

[41] A. Harimaya, T. Himi, N. Fujii et al., "Induction of CD69 expression and Th1 cytokines release from human peripheral blood lymphocytes after *in vitro* stimulation with *Alloiococcus otitidis* and three middle ear pathogens," *FEMS Immunology and Medical Microbiology*, vol. 43, no. 3, pp. 385–392, 2005.

[42] K. LeMessurier, H. Häcker, E. Tuomanen, and V. Redecke, "Inhibition of T cells provides protection against early invasive pneumococcal disease," *Infection and Immunity*, vol. 78, no. 12, pp. 5287–5294, 2010.

[43] J. Vega-Ramos, E. Alari-Pahissa, J. D. Valle et al., "CD69 limits early inflammatory diseases associated with immune response to *Listeria monocytogenes* infection," *Immunology and Cell Biology*, vol. 88, no. 7, pp. 707–715, 2010.

[44] K. Radulovic, C. Manta, V. Rossini et al., "CD69 regulates type I IFN-induced tolerogenic signals to mucosal CD4 T cells that attenuate their colitogenic potential," *The Journal of Immunology*, vol. 188, no. 4, pp. 2001–2013, 2012.

Hindawi Publishing Corporation
Journal of Immunology Research
Volume 2016, Article ID 1958650, 12 pages
http://dx.doi.org/10.1155/2016/1958650

Review Article

Molecular Mechanisms of Induction of Tolerant and Tolerogenic Intestinal Dendritic Cells in Mice

Alex Steimle and Julia-Stefanie Frick

University of Tübingen, Institute of Medical Microbiology and Hygiene, Elfriede-Aulhorn-Strasse 6, 72076 Tübingen, Germany

Correspondence should be addressed to Julia-Stefanie Frick; julia-stefanie.frick@med.uni-tuebingen.de

Received 22 October 2015; Revised 6 January 2016; Accepted 17 January 2016

Academic Editor: Silvia Beatriz Boscardin

How does the host manage to tolerate its own intestinal microbiota? A simple question leading to complicated answers. In order to maintain balanced immune responses in the intestine, the host immune system must tolerate commensal bacteria in the gut while it has to simultaneously keep the ability to fight pathogens and to clear infections. If this tender equilibrium is disturbed, severe chronic inflammatory reactions can result. Tolerogenic intestinal dendritic cells fulfil a crucial role in balancing immune responses and therefore creating homeostatic conditions and preventing from uncontrolled inflammation. Although several dendritic cell subsets have already been characterized to play a pivotal role in this process, less is known about definite molecular mechanisms of how intestinal dendritic cells are converted into tolerogenic ones. Here we review how gut commensal bacteria interact with intestinal dendritic cells and why this bacteria-host cell interaction is crucial for induction of dendritic cell tolerance in the intestine. Hereby, different commensal bacteria can have distinct effects on the phenotype of intestinal dendritic cells and these effects are mainly mediated by impacting toll-like receptor signalling in dendritic cells.

1. Introduction

The mammalian intestinal immune system has to rise to different challenges. On the one hand, it has to tolerate the intestinal microbiota consisting of commensal bacteria, fungi, and other microbes, thereby profiting from beneficial bacterial metabolites and other advantages. On the other hand, pathogen induced infections of the intestine have to be cleared without spacious damage of the intestinal tissue. Since a loss of tolerance to the own microbiota causes chronic inflammation of the gut, efficient sensing of the intestinal homeostasis is crucial to avoid pathophysiological immune responses. In this context, intestinal tolerogenic dendritic cells play a crucial role as key mediators for the maintenance of the intestinal homeostasis. While the main question "how does the host manage to tolerate its own intestinal microbiota?" is pretty simple, the answer is not trivial.

Here, we want to focus on (1) the molecular mechanisms that might contribute to the induction of tolerogenic DCs in the intestine and (2) the potential clinical applications arising from these findings for the treatment of chronic inflammatory disorders of the gut: inflammatory bowel diseases.

2. Intestinal Dendritic Cells: Subsets and Biological Functions

Dendritic cells (DCs) comprise a heterogeneous leukocyte population of different developmental origin and with distinct surface markers and biological functions. DCs originate from blood monocytes or a common DC progenitor in the bone marrow under steady-state conditions. The differentiation into DCs relies on local presence of GM-CSF [1]. DCs in general are utterly specialized antigen presenting cells (APCs) which are able to induce a variety of different immune responses. They are the most important cell type connecting the innate immune system with adaptive immune responses [2]. DCs patrol almost all lymphoid and nonlymphoid organs and meld properties of the innate and adaptive immunity and therefore link these two mechanistically distinct branches of the immune system [3]. Furthermore, DCs play a pivotal role in mediating a protective adaptive immunity against

pathogens while maintaining immune tolerance to self-antigens. Their crucial role for mediating self-tolerance is confirmed by the observation that DC depletion leads to a loss of self-tolerance and results in myeloid inflammation and the induction of autoimmune processes [4].

The gut-associated lymphoid tissue (GALT) is the largest immune organ of the body. The GALT has to ensure that there is a dynamic balance between protective immunity by fighting pathogens and regulatory mechanisms to prevent autoimmunity [5]. Since the GALT is constantly exposed to large amounts of luminal antigens like food metabolites, foreign pathogens, and commensal microbes, this balance has to be well adjusted in order to create homeostatic conditions in the intestine. Dendritic cells are hereby the key players for maintaining intestinal homeostasis [6]. They are spread out in the connective tissue underlying the epithelial layer of the gut [7].

2.1. Morphological Differences between DCs and Macrophages (MΦ) in the Murine Intestine. DCs belong to the group of mononuclear phagocytes (MPs) with macrophages (MΦ) being another cell type belonging to this group. Discrimination between DCs on one hand and MΦ on the other hand is still a matter of ongoing debate. However, concerning intestinal DCs and MΦ, certain surface markers and transcription factors have been reported to be uniquely expressed by only one of these two groups. In the murine intestine, surface proteins which are exclusively expressed by DCs are CD103 [8–10], CD26, and CD272 [9]. However, CD103 is not expressed from every DC subset (see below) [11–13]. A DC specific transcription factor is Zbtb46 [13]. The only MPs in the murine intestine that express the proteins CD14, MerTK [9, 14], F4/80, and CD64 [15] are intestinal MΦ. The widely used surface markers for DC-macrophage discrimination, CD11c and MHC-II, are not useful to distinguish murine intestinal DCs from MΦ, since both proteins can be expressed in DC or macrophage subpopulations [13, 15–19]. The expression of CD11b and MHC-II varies among DC and MΦ subpopulations [13]. Therefore, the protein expression pattern of murine intestinal DCs under steady state conditions can be summarized as $CD11c^{+}CD103^{+/-}CD11b^{+/-}$MHC-$II^{+}CD26^{+}CD272^{+}Zbtb46^{+}CD14^{-}MerTK^{-}F4/80^{-}CD64^{-}$, while the phenotype of intestinal murine MΦ is $CD11c^{+/-}CD103^{-}CD11b^{+/-}$MHC-$II^{+/-}CD26^{-}CD272^{-}Zbtb46^{-}CD14^{+}MerTK^{+}F4/80^{+}CD64^{+}$. Another distinctive feature between DCs and MΦ is the migratory and proliferation behaviour. In general, intestinal DCs are short-lived, proliferating migratory cells while MΦ are tissue resident, long-lived, and nonproliferating [13].

2.2. DC Subpopulations in the Intestine. As mentioned above, dendritic cells do not comprise a homogenous cell population. Different ways to distinguish one DC from another are published and popular. The most prominent approach to differentiate between distinguishable DC subsets is to focus on different expression of surface proteins, especially CD103 and CD11b [12, 13]. However, Guilliams and van de Laar have recently proposed to distinguish DCs rather by their biological function and cellular origin than their surface marker expression [11, 12]. We will adapt this system, but we will focus on DC subsets located in the intestine and add latest findings on different surface marker expression among these subsets [13]. In general, DCs derive from common dendritic cell progenitors (CDP) which, in turn, develop from hematopoietic precursor cells. CDPs may differentiate into either preplasmacytoid DCs (pre-pDCs) or precommon DCs (pre-cDCs) precursor cells [20, 21]. Murine pDCs are characterized by PDCA1 expression and their development is dependent on the transcription factors BATF3 [22], ID2 [23], NFIL3 [24], E2-2 [25], and IRF8 [22]. Murine cDCs commonly express XCR1 and SIRPα [26] and need RelB [27], RPBJ [28], and IRF4 [29] for differentiation. Intestinal murine cDCs additionally express CD103 and can be further subdivided into two ontogenetically different populations, dependent on their surface expression of CD11b [30]. IRF4 is needed for the $CD11b^{+}$ lineage of these CD103-expressing or CD103-nonexpressing conventional DCs [29, 31, 32]. $CD103^{+}CD11b^{-}$ build up cDC subset 1 (cDC1) whereas $CD103^{+}CD11b^{+}$ form subgroup cDC2 (see Figure 1). One of the most important events for the maintenance of intestinal homeostasis is the induction of regulatory T cells (Tregs) (see below). Besides TGF-β, Treg formation is dependent on the presence of retinoic acid (RA) that is produced by dendritic cells [33, 34]. But only DCs possessing retinaldehyde dehydrogenases (ALDHs) can convert vitamin A-derived retinol to RA. Therefore, ALDH is a crucial enzyme for a subsequent induction of Tregs and thus promotion of intestinal tolerance and homeostasis. It was not clear which $CD103^{+}$ DC subset is responsible for Treg induction, since both $CD103^{+}CD11b^{+}$ and $CD103^{+}CD11b^{-}$ DCs can produce RA and induce Tregs *in vitro* [35, 36]. Meanwhile, it could be demonstrated that each $CD103^{+}$ DC subset ($CD11b^{+}$ versus $CD11b^{-}$) can be subdivided in an ALDH-expressing and a non-ALDH-expressing subset [35]. Therefore, both $CD11b^{+}CD103^{+}$ and $CD11b^{-}CD103^{+}$ DCs are able to induce a RA-mediated Treg formation. This was initially demonstrated in skin-draining lymph nodes [35], but Janelsins et al. confirmed the presence of $CD103^{+}CD11b^{+}ALDH^{+/-}$ DCs also in the murine cLP [37]. Both $CD103^{+}$ DC subsets together monitor the luminal environment in the intestine. Not only are $CD103^{+}$ DCs able to induce Treg-mediated immune tolerance in the intestine but they are also able to promote Th17 differentiation of naïve T cells. Th17 cells contribute to the manifestation of autoimmune diseases [38, 39] and $CD103^{+}CD11b^{+}$ seem to be more efficient in Th17 promotion than their $CD11b^{-}$ counterparts [36, 40]. It can be assumed that $ALDH^{-}CD103^{+}CD11b^{+/-}$ might promote this Th17 immune response, but final evidence is missing until now.

Concerning their distribution in the intestine, $CD103^{+}CD11b^{-}$ DCs are prominent in the colonic lamina propria (cLP), while $CD103^{+}CD11b^{+}$ DCs are mostly found in the LP of the small intestine [29]. Additionally, $CD103^{+}CD11b^{+}$ DCs from the mesenteric lymph nodes (MLN) also express ALDH, which is, surprisingly, abdicable for the induction of Tregs since a loss of ALDH activity in the MLN did not affect Treg induction [29]. This might support the hypothesis that

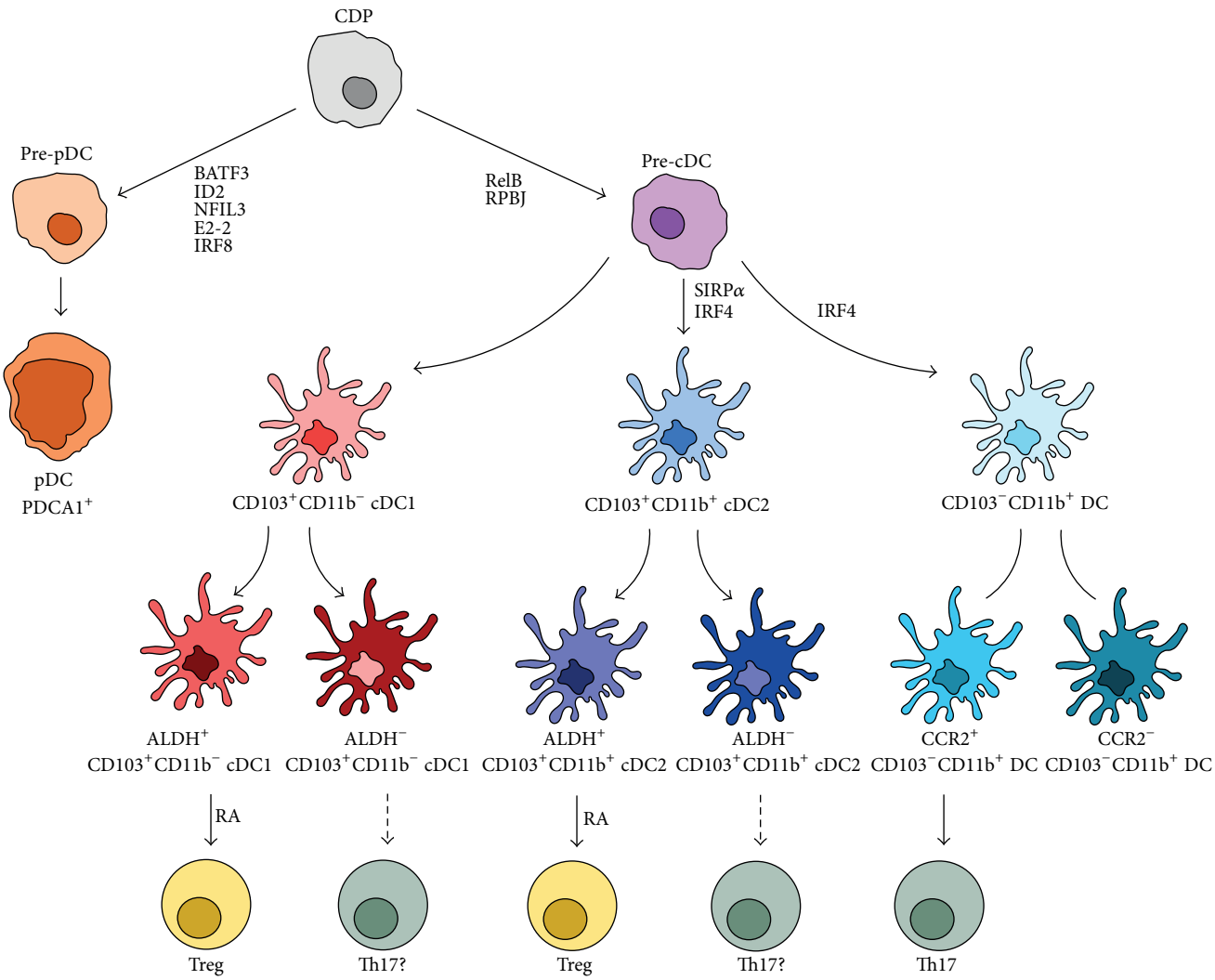


Figure 1: Ontogenetic development of intestinal dendritic cells subpopulations. Adapted from Guilliams et al. [35] and expanded by findings from Scott et al. [13]. See text for details. Common dendritic cell progenitor (CDP), preplasmacytoid dendritic cell (pre-pDC), precommon dendritic cell (pre-cDC), plasmacytoid dendritic cell (pDC), common dendritic cell (cDC), aldehyde dehydrogenase (ALDH), regulatory T cell (Treg), retinoic acid (RA), and T-helper 17 cell (Th17). Arrows with solid lines represent published data, and arrows with broken lines represent speculative hypotheses with missing final evidence.

ALDH activity is more important at other intestinal sites like the lamina propria of the small or large intestine for later Treg induction after DC migration.

Recently, Scott et al. discovered an additional CD103-negative DC population in the murine intestine [13]. There is a $CD11c^+MHC\text{-}II^+CD103^-CD11b^+$ cell population, of which about 15% provide features of DCs like Zbtb64, CD26, and CD272 expression; they respond to Flt3L; they are migratory cells and lack macrophage markers like F4/80 and CD64 [13, 41]. They could be shown to be derived from committed pre-DCs as are $CD103^+$ mucosal DCs [8]. These $CD103^-$ DCs can be further subdivided into two functionally distinct subpopulations dependent on their CCR2 expression. $CCR2^+CD103^+CD11b^+$ DCs are more efficient in Th17 induction than their CCR-negative counterparts and a loss of the $CCR2^+CD103^-CD11b^+$ DCs leads to a defective Th17 response and therefore fails to clear a *Citrobacter* infection *in vivo*.

Another specific protein that is expressed exclusively by intestinal DCs and not by intestinal MΦ or nonintestinal DCs is SIRPα [42, 43]. It seems to be essential for the generation of $CD103^+CD11b^+$ since a loss of function of SIRPα results in a decrease of this DC population in the intestine, accompanied by markedly reduced induction of Th17 immune responses under steady-state and inflammatory conditions [42].

In general, it is important to keep in mind that CD103 expression on DCs is not a marker for universal tolerogenicity, since (1) even $CD103^+$ DCs can fail to induce tolerogenicity if ALDH is not expressed and (2) a tolerogenic environment can be established even in the absence of $CD103^+$ DCs [44].

2.3. Locations of Intestinal DCs. The murine intestine is a multifarious habitat for DCs where distinct sites harbour different DC subsets. A common feature of intestinal DCs distinguishing them from DCs from other nonintestinal tissues

is the expression of CD103, with the already mentioned exception of $CCR2^{+/-}CD103^{-}CD11b^{+}$ DCs, especially DCs in the small intestine (SI) and Peyer's Patches (PP) in mesenteric lymph nodes (MLN) and, with minor occurrence, in the colonic lamina propria (cLP) [8, 29, 41, 43, 45]. DCs from nonlymph node tissues remain some days at their inherent site before migrating to neighbouring draining lymph nodes [35, 46].

3. Antigen Sensing and Sampling by Intestinal Dendritic Cells

Invading microorganisms are recognized by pattern recognition receptors (PRRs) on the DC surface. PRRs include toll-like receptors (TLRs), retinoic acid-inducible gene I-like receptors (RLRs), and nucleotide-binding oligomerization domain-like receptors (NLRs) [47, 48]. PRRs recognize pathogen-associated molecular patterns (PAMPs) [49]. PAMPs comprise a heterogeneous class of different antigens, that is, surface components of bacteria. One of the most prominent PAMPs which usually induces DC maturation is lipopolysaccharide (LPS), an integral cell surface component of all Gram-negative bacteria. Usually, dendritic cells underlie the intestinal epithelium and therefore the connection to the colonic lumen is restricted. However, there are three prominent ways how intestinal dendritic cells can sample luminal antigens: (1) with participation of goblet cells which deliver soluble and preferably low molecular weight antigen to neighbouring DCs [50], (2) with the support of $CX3CR1^{+}$ phagocytotic cells which can actively capture antigen followed by transport to neighbouring DCs via tight junctions [51], and (3) a direct sampling by DCs that extend their dendrites towards the lumen establishing a direct connection to the colonic lumen [52].

4. Intestinal Dendritic Cells and the Gut Microbiota

$CD103^{+}$ DCs are reported to sample mainly bacteria [52] in contrast to $CX3CR1^{+}$ MΦ which also capture soluble proteins and fungi [51, 53]. This illustrates the relevance of the bacterial microbiota composition for intestinal DCs. Interaction of DCs with the gut microbiota can occur directly by sampling bacterial antigen or by interaction with bacterial metabolic products like short chain fatty acids (SCFAs). SCFAs like butyrate can interact with the DC receptor GPR109A which finally leads to an IL-10 mediated induction of Tregs [54]. Since not all gut commensal bacteria produce SCFAs, a microbiota shift leading to dysbiosis can profoundly affect immunological mechanisms in the intestine. Toll-like receptor (TLR) signalling in DCs also seems to be crucial for the maintenance of intestinal homeostasis. Different bacterial components bind to distinct TLRs on the surface of DCs resulting in the activation of intracellular signalling cascades which leads to DC maturation or semimaturation (see below) accompanied by secretion of pro- or anti-inflammatory cytokines. The TLT adaptor molecule TNF-receptor associated factor 6 (TRAF6) seems to play a pivotal role in maintaining intestinal homeostasis since $Traf6^{-/-}$ mice fail to maintain intestinal homeostasis mediated by a reduction of Tregs and an increase of T-helper 2 (Th2) cells, finally resulting in a microbiota composition-dependent induction of colonic inflammation [55].

5. The Different Maturation Phenotypes of Dendritic Cells

The capability of initiating an immune response depends on the current DC maturation state. Usually, antigen encounter results in rapid DC maturation which is characterized by efficient endocytosis and antigen processing. Furthermore, upregulation of MHC-II and T cell costimulatory molecules like CD40, CD80, and CD86 enhanced expression of chemokine receptors and the secretion of proinflammatory cytokines like IL-1β, IL-6, TNFα, and IL-12 are part of DC maturation. These events influence and activate other cellular components of an induced immune response like MΦ, neutrophils, and especially T cells [56].

5.1. Mature DCs (mDCs). Induction of DC maturation is accompanied by a loss of the capacity to take up and process antigen [57]. However, they literally develop into professional antigen presenting cells (APCs) indicated by powerful antigen presentation to naïve T cells [2], as well as by their ability to migrate to secondary lymphatic organs where they present antigens to T cells.

5.2. Immature DCs (iDCs). Immature DCs (iDCs) express low amounts of MHC-II and T cell costimulatory molecules. They tend to promote T cell anergy and to generate Tregs, with both effects being crucial for intestinal homeostasis [58]. iDCs furthermore express high levels of PRRs with which they mediate the recognition of potential antigens and therefore their endocytosis [57].

5.3. Semimature DCs (smDCs) and Tolerant DCs. The definition of a semimature DC phenotype is less distinct. The most important property of smDCs uniting different definitions is the inability to induce a proinflammatory Th1 or Th17 response and to be nonresponsive, or in other words "tolerant," towards subsequent maturation stimuli [59, 60] with the latter being the criterion that mediates the tolerogenic functions of smDCs [60]. DC semimaturation leads to a certain expression of T cell activation and a cytokine secretion pattern that is distinct from the ones of immature and mature DCs. The definite phenotype varies from one semimaturation inducing strategy to another. SmDCs that are generated by treating immature DCs with TNFα display a phenotype that can be summarized as $CD11c^{+}$ $MHCII^{hi}CD86^{hi}CD80^{hi}CD40^{lo}CD54^{+}CD205^{hi}CD25^{hi}TNF^{lo}$ $IL\text{-}12p40^{lo}IL\text{-}10^{lo}$ [61]. Induction of semimaturation via low-dose LPS and subsequent dexamethasone treatment results in $CD14^{+}CD1a^{lo}CD80^{hi}CD86^{hi}MHCII^{hi}IL\text{-}10^{hi}TNF^{lo}$ DCs [62]. We use a Gram-negative gut commensal, *Bacteroides vulgatus*, to induce semimaturation and define the smDC

Table 1: Phenotypes of semimature dendritic cells dependent on semimaturation inducing agent. LPS (lipopolysaccharide), Dex (dexamethasone), and PSA (polysaccharide A); high expression (hi), low expression (lo), intermediate expression (int), and not determined (n.d.).

Semimaturation inducing agent	MHC-II	CD40	CD80	CD86	IL-10	IL-6	TNFα	IL-12	Source
B. vulgatus	int	lo	lo	lo	n.d.	int	lo	lo	[59]
TNFα	hi	lo	hi	hi	lo	n.d.	lo	lo	[61]
LPS + Dex	hi	n.d.	hi	hi	hi	n.d.	lo	n.d.	[62]
B. fragilis PSA	int	n.d.	n.d.	n.d.	hi	n.d.	n.d.	n.d.	[66]
ATP + LPS	hi	lo	hi	hi	hi	n.d.	lo	lo	[120]
E. multilocularis	lo	n.d.	n.d.	lo	int	lo	n.d.	lo	[121]
Low dose LPS	int	lo	lo	lo	n.d.	int	lo	lo	[64]
α-1 Antitrypsin	int	lo	n.d.	int	hi	lo	n.d.	n.d.	[65]

phenotype as $CD11c^+MHCII^{int}CD40^{lo}CD80^{lo}CD86^{lo}$ $TNF\alpha^{lo}IL\text{-}12^{lo}IL\text{-}6^{int}$ [59]. Besides these strategies, DC semimaturation can be induced by treating immature DCs with ATP and LPS [63], low dose *Salmonella* LPS [64], α-1 antitrypsin [65], *Bacteroides fragilis* PSA [66], or *Echinococcus multilocularis* cell aggregates [67]. The resulting phenotypes concerning the most important immunomodulatory molecules are summarized in Table 1.

5.4. Tolerogenic DCs. While mature DCs (mDCs) promote efficient induction of inflammatory immune responses, iDCs and smDCs fail to do so. They rather have the property to actively prevent from inflammatory reactions and are therefore also termed tolerogenic DCs (tolDCs). The term "tolerogenic" includes one, several, or all of the following features DCs must provide to be considered "tolerogenic": (1) the induction of unresponsiveness of T cells, (2) active induction of Tregs, (3) inhibition of proinflammatory T cell responses, and (4) promotion of T cell apoptosis or T cell anergy [6]. In this context, the interplay between the intestinal epithelial cells and the host immune system is of essential importance.

More generally, regulatory or tolerogenic DCs keep their ability to present antigens, but at the same time they downregulate the expression of T cell costimulatory molecules and proinflammatory cytokines but in turn upregulate inhibitory molecules like PD-L1, CD95L, or IDO as well as anti-inflammatory cytokines such as TGF-β and IL-10 [68]. Furthermore, they are resistant to a second maturation inducing signal [68]. Importantly, DCs also influence the differentiation of naïve T cells into Th1, Th2, Th17, or Treg cells, mostly due to supplying a certain cytokine environment [69]. In a healthy individual, the presence of tolDCs is important and a loss of tolDCs can result in the development of AID [4]. Semimature DCs are potent tolerant and tolerogenic DCs since they fulfil many to all of the above-mentioned criteria, dependent on the agent with which semimaturation is induced. As already mentioned before, the main characteristic that makes semimature DCs tolerogenic is their unresponsiveness (tolerance) towards subsequent maturating stimuli [59, 64, 65].

6. The Role of Dendritic Cells in Induction of Inflammatory Bowel Disease (IBD)

The development of inflammatory bowel diseases (IBD) with its two major representatives Crohn's disease (CD) and ulcerative colitis (UC) is associated with (1) an inappropriate immune response to normally benign stimuli like commensal microbes, (2) an inefficient clearance of microbes leading to a continuous stimulation of the immune system, or (3) failing to turn from an adequate proinflammatory response to inflammation resolving anti-inflammatory immune reactions [70]. In this context, the composition of the intestinal microbiota is decisive for the onset of colonic inflammation in most mouse models of experimental colitis [71] and intestinal DCs are crucial for driving immune responses in either a proinflammatory or a rather homeostatic direction [72]. For example, $Il10^{-/-}$ mice develop chronic colitis which results from the absence of suppression of MyD88-dependent commensal-induced inflammation by IL-10 [73].

Under steady-state conditions, circulating $Ly6C^{hi}$ monocytes are repopulated into tolerogenic $F4/80^{low}CD103^+$ $CD11c^+$ LP DCs, which contribute to homeostasis by supporting tolerogenic functions [16]. On the other hand, under inflammatory conditions during colitis, $Ly6C^{hi}$ monocytes develop into $CD103^-CX3CR1^{int}CD11b^+$ LP DCs, which mediate inflammation during colitis [16].

The tolerogenic functions of intestinal DCs are mainly mediated by the induction of regulatory T cells (Tregs). As a characteristic feature, Tregs express the transcription factor forkhead box P3 (Foxp3) [74]. Induction of $CD4^+CD25^+Foxp3^+$ Tregs is essential for intestinal homeostasis [75] and a loss of Tregs leads to a fatal multiple-organ-associated autoimmune disease [76]. Tregs are usually converted in the peripheral immune system with the help of $CD103^+$ dendritic cells [77] whereupon this Treg induction is dependent on the presence of TGF-β and retinoic acid (RA) [78].

However, during colitis, $CD103^-CXCR1^{int}CD11b^+$ DCs, although also present under steady-state conditions, massively infiltrate the colonic LP and mediate proinflammatory

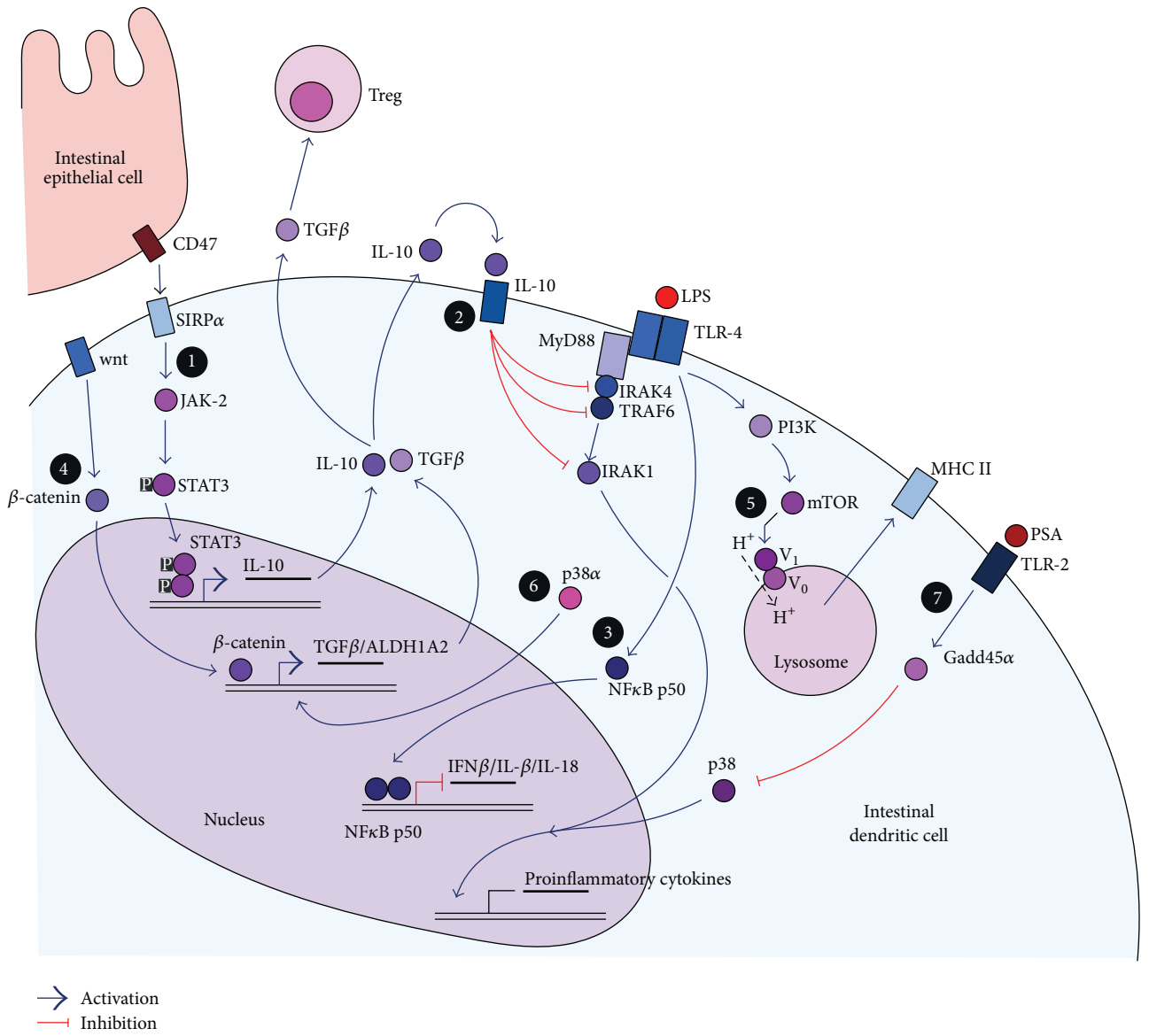


FIGURE 2: Possible molecular mechanisms of tolerance induction in intestinal dendritic cells. The white numbers in black circles refer to the numbering of regulation mechanisms in the text. See text for details.

immune responses by producing IL-12, IL-23, iNOS, and TNF [16].

7. Possible Molecular Mechanisms of DC Tolerance Induction in the Intestine

Less is known about defined mechanisms of tolerance induction in intestinal DCs. However, knowledge about tolerance induction mechanisms of other DC subsets or of *in vitro* generated DCs can be transferred to intestinal DCs to explain how they manage to tolerate luminal bacterial or food antigens and therefore prevent from uncontrolled inflammatory reactions. Here, we want to present latest research results and discuss how and if these findings can be assigned also to intestinal DCs. All proposed mechanisms are summarized in Figure 2.

(1) Cell-to-Cell-Contact and STAT3 Signaling. Epithelial cells of the intestine express the surface protein CD47 which can directly interact with signal regulatory protein α (SIRPα) expressed on the surface of DCs which underlie the intestinal epithelial cell layer. This protein-protein-interaction has been shown to result in a janus kinase-2 (JAK-2) dependent signal transducer and activator of transcription 3 (STAT3) activation downstream of SIRPα in DCs [79]. STAT3 activation, in turn, leads to enhanced IL-10 secretion from DCs and therefore promotes tolerogenic properties in the intestinal environment [79]. STAT3 has long been known as a crucial negative regulator of immunity. Disruption of STAT3 leads to

a loss of T cell tolerance in mice and efficient STAT3 signaling is associated with the immature DC phenotype, general IL-10 secretion, and tolerance induction [80]. Therefore, not only does DC alone seem to be important for homeostasis maintenance but also the "teamwork" with neighbouring epithelial cells seems to contribute to tolerance mechanisms.

(2) IL-10 as a Central Cytokine for Intestinal Homeostasis. Interleukin-10 is a key inhibitory cytokine in T cell activation and a mediator of intestinal homeostasis [81]. It is secreted by T cells, B cells, and most myeloid-derived cells [82]. Mice lacking functional IL-10 or its IL-10R receptor counterpart spontaneously develop severe intestinal inflammation.

Supporting the idea of IL-10 being a crucial mediator for intestinal homeostasis [83]. Also humans with defective mutations in the genes encoding for IL-10 or IL-10R develop a severe form of enterocolitis within the first months after birth [84]. These observations made IL-10 a promising therapeutic candidate in order to treat chronic inflammatory conditions of the intestine. However, results were not convincing since, in mice as well as in humans, IL-10 administration did not ameliorate the inflammatory conditions [85]. IL-10 not only affects T cell responses but can also provide autocrine and paracrine effects on DCs. Since DCs express the IL-10 receptor (IL10R), IL-10 can bind to IL10R resulting in a negative regulation of myeloid differentiation primary response 88 (MyD88) signaling inside DCs. MyD88 is an adaptor molecule of TLRs and is required for downstream TLR signalling. IL-10/IL10R interaction mediates this negative regulation by a downregulation of interleukin 1 receptor associated kinase 4 (IRAK4) on the protein level without altering IRAK4 gene transcription rates [86]. It also leads to dissociation of MyD88 from TLRs and subsequently promotes proteasomal degradation of IRAK1, IRAK4, and TRAF6, therefore silencing MyD88-dependent TLR signalling [86]. However, this is just the case if LPS as a TLR4 ligand is present at the same time to induce TLR signaling. IL-10 silencing of MyD88 signaling seems to be crucial for the maintenance of intestinal homeostasis since *IL-10*$^{-/-}$ mice fail to develop intestinal inflammation if these mice simultaneously lack MyD88 [73]. As a consequence, the cytokine environment does also affect DCs in their ability to induce tolerance mechanisms.

(3) NFκB Signalling as a Mediator for Tolerogenicity. A key regulator for DC maturation and inflammatory reactions in general is NFκB [87, 88]. NFκB family members do not only have an activating potential for the induction of proinflammatory cytokines. Two NFκB proteins, p50 and p52, have been associated with transcription repression functions and therefore induction of tolerance [89, 90]. Both proteins lack the carboxyterminal transactivation domain and can form inhibitory homodimer complexes that prevent from transcription of proinflammatory genes. NFκB p50 has been shown to promote a tolerogenic DC phenotype by negatively affecting DC survival and their capacity to efficiently activate T cells [91]. Accumulation of p50 in the nucleus of tolerogenic DCs can be accompanied by enhanced expression of tolerance-promoting molecules like indoleamine dioxygenases (IDOs) and decreased expression of proinflammatory cytokines like IFNβ, IL-1β, and IL-18 [91]. These implications for p50 in the induction of tolDCs are supported by the finding that p50-deficient DCs are weak inducers of a Foxp3$^+$ Treg differentiation [92]. Formation of p50-p50 homodimers contributes to LPS tolerance in MΦ [93] and p50 expression in immature DCs is crucial to prevent from autoreactive T cells [91].

(4) β-Catenin Promotes DC Tolerogenicity. β-catenin is a transcription factor and part of the wnt signalling pathway. It could be demonstrated that this signalling pathway with the subsequent release of β-catenin into the nucleus results in the induction of tolDCs [94]. Gene expression profiles of intestinal LP DCs revealed that this signalling pathway is decisive for the DC to become either mature or tolerogenic. β-catenin translocation into the DC nucleus resulted in the expression of various tolerance-associated factors like retinoic acid-metabolizing enzymes, IL-10 and TGF-β [94].

(5) Prevention of V-ATPase Domain Assembly Induces Tolerogenic DCs. Vacuolar (H^+)-ATPases (V-ATPases) are ATP-driven proton pumps. They are composed of two domains: a peripheral V_1 domain and membrane-embedded V_0 domain [95]. V-ATPases are involved in acidification of lysosomes by shuffling protons from the cytosol into the lysosomic lumen [96]. The pH value of lysosomes is a crucial regulator for the efficiency of antigen processing since lysosomal proteases being involved in antigen proteolysis require acidic environments [95]. The most important mechanism to regulate lysosomal acidification is to control the assembly of the two V-ATPase domains which is a required event for forming a functional proton pump. It is known that activation of TLRs promotes domain assembly and therefore supports DC maturation [96]. Domain assembly seems to be a PI-3 kinase and mTOR mediated event since inhibitory substances for both molecules could block V-ATPase domain assembly and therefore prevent from DC maturation and promote the induction of a tolerogenic phenotype [95]. Also, stimulation of integrins and E-cadherins by cluster disruption of DC prevents from domain assembly and supports induction of a tolerogenic phenotype [96, 97].

(6) p38α Expression Influences Expression of ALDH1A2. MAP kinases like ERK, JNK, and p38α form central pathways that are activated by innate immune signals like PAMPs [98, 99] and excessive activation of MAP kinases are reported to be associated with many autoimmune and inflammatory diseases [99]. However, the MAP kinase p38α provides a dichotomic role. Besides being involved in promoting proinflammatory responses, its activity seems also to be crucial for the induction of a tolerogenic phenotype in intestinal CD103$^+$ DCs. In these DCs, p38α is constitutively active and this activity is crucial for the expression of TGF-β and aldehyde dehydrogenase 1A2 (ALDH1A2), the latter being involved in metabolizing retinoic acid (RA). TGF-β and RA are involved in Treg generation and therefore promote gut homing properties of T cells [99].

(7) Gadd45α-Mediated TLR2 Signalling Contributes to Tolerogenic Features of Intestinal DCs. An abundant bacterial gut commensal, *B. fragilis*, is able to protect from the induction of EAE and experimental colitis and increases the proportions of $CD103^+CD11c^+$ DCs [100, 101]. It could be demonstrated that this effect is mediated by polysaccharide A (PSA), an immunomodulatory component present in outer membrane vesicles derived from *B. fragilis* bacteria [66]. PSA promotes immunological tolerance by inducing IL-10 producing $Foxp3^+$ Tregs and protects animals from experimental colitis [102]. The PSA caused induction of tolDCs is dependent on TLR2 and growth arrest and DNA-damage-inducible 45 α (Gadd45α), since Gadd45α-deficient DCs are unable to mediate PSA-induced protection of experimental colitis [66]. Gadd45α itself inhibits an alternative way of MAPK p38-mediated signalling [103] and PSA-containing outer membrane vesicles lead to upregulation of Gadd45α [66].

Taken together, all of the mentioned molecular mechanisms of tolerance induction in DCs are potentially able to take place in the intestine, either through participation of neighbouring intestinal epithelial cells or through direct interaction of DCs with luminal content. Concerning luminal content, bacteria and their PAMPs could promote all of the potential mechanisms via interaction with host pattern PRRs, especially TLRs. We identified apathogenic Gram-negative commensal strains, namely, *Bacteroides vulgatus* mpk and *Escherichia coli* mpk, mediating completely contrary effects on DC maturation and, in consequence, the progress of experimental colitis in mice [104, 105]. As mentioned above, *B. vulgatus* interaction with immature DCs converted them into a tolerant and tolerogenic semimature phenotype characterized by low to intermediate expression of MHC-II, CD40, CD80, and CD86, almost absent secretion rates of TNFα and IL-12p70, and remarkable IL-6 secretion [59]. As a characteristic of tolerant DC, this phenotype could not be overcome with a subsequent maturating bacterial stimulus or by CD40 ligation [106]. *E. coli* mpk stimulation, however, resulted in efficient DC maturation. As a consequence, *E. coli* mpk colonization in experimental mouse colitis using $Il2^{-/-}$ mice resulted in colonic inflammation, a feature that could be prevented by simultaneous *B. vulgatus* mpk colonization [104, 105]. We could prove that both bacteria differentially alter the phenotype of dendritic cells not only *in vitro* but also *in vivo* in the colonic LP via adjusted bacterial colonization of the gut [105]. In this context, feeding *B. vulgatus* always induced tolerant and tolerogenic DC in the colonic LP. In another study using distinct *E. coli* bacteria that just differ in the structure of their cell surface LPS, we could prove that the LPS structure alone decides if LP DCs are converted into a mature phenotype and therefore promote inflammation or if they are converted into tolerogenic semimature DCs and thus maintain intestinal homeostasis [107]. As LPS primarily signals via TLR4, tolerance induction mechanisms where NFκB p50, Gadd45α, MyD88-signaling, β-catenin, and/or V-ATPase domain assembly are involved could be possible. Since LPS is a cell wall component of all Gram-negative bacteria, the resulting abundance in the intestinal lumen could largely contribute to tolerance induction in intestinal DCs. As we have demonstrated, different commensal bacteria can have opposite effects on DC maturation. This makes the composition of the microbiota decisive on whether DCs mediate tolerogenic or inflammatory LPS-triggered immune responses.

8. Perspectives for Clinical Approaches for the Treatment of IBD Using Tolerant and Tolerogenic Dendritic Cells

In order to be suitable as an administrable therapeutic, tolerogenic DCs (tolDCs) have to be generated *in vitro*. One efficient way to induce tolDCs is coincubating them with apoptotic cells. Phagocytosis of apoptotic cells through DCs results in production of TGF-β which in turn contributes to immune tolerance. Apoptotic cell-treated DCs efficiently converted naïve $CD4^+$ T cells into $Foxp3^+$ Tregs [108, 109]. In general, apoptotic cell induced tolDCs are important for induction of immune tolerance [110, 111]. They provide upregulation of Galectin-1 and CD205 [112], two molecules that facilitate the manifestation of immune tolerance [113, 114]. At the same time, apoptotic cell treated DCs downregulate Gr-1 and B-220 [112], two molecules triggering inflammatory responses. These DCs furthermore downregulate the transcription factor RORγt which is the decisive transcription factor for Th17 differentiation. Not only does treatment with apoptotic cells lead to induction of tolDCs but also treatment with herbal coumarins [115] and the macrocyclic antibiotic rapamycin [116] leads to tolDC induction. *In vitro* generated tolDCs have already been successfully used for the treatment of autoimmune disorders in animal models and peripheral tolerance could be restored by administrating tolerogenic DCs [117]. Approaches to treat autoimmune type 1 diabetes in a mouse model using nonobese diabetic (NOD) mice with tolerogenic DCs were very successful [112]. To do so, apoptotic islet cells were used to induce DC mediated tolerance against own islet cells [118]. All these applications lead to the question if transfer of tolerogenic dendritic cells would also be an approach to treat IBD and, if yes, which method to induce DC tolerance would be the method of choice. A published approach for the treatment of Crohn's disease patients is *in vitro* generation of DCs followed by pulsing with dexamethasone, proinflammatory cytokines IL-6, IL-1β, and TNFα, and PGE_2 [119]. Concerning our findings that a certain gut commensal, *B. vulgatus* mpk, efficiently induces tolerant and tolerogenic DCs *in vitro* as well as *in vivo* [59, 104, 105], we would recommend using host gut commensal bacteria for *in vitro* tolDC generation. In order to provide more potential luminal antigens presented by MHC-II of tolDCs, a defined mixture of commensal bacteria could be used. This would enlarge the amount of antigenic peptides against which tolerance would be induced.

Disclosure

The authors disclose all commercial affiliations and competing financial interests.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

Work by Julia-Stefanie Frick was supported by the DFG (DFG FR 2087/6-1, DFG FR 2087/8-1, CRC685, SPP1656), the DFG research training group 1708, the Bundesministerium für Bildung und Forschung (BMBF), and the German Centre for Infection Research (DZIF).

References

[1] S. Rutella, G. Bonanno, L. Pierelli et al., "Granulocyte colony-stimulating factor promotes the generation of regulatory DC through induction of IL-10 and IFN-α," *European Journal of Immunology*, vol. 34, no. 5, pp. 1291–1302, 2004.

[2] A. Iwasaki and R. Medzhitov, "Regulation of adaptive immunity by the innate immune system," *Science*, vol. 327, no. 5963, pp. 291–295, 2010.

[3] R. M. Steinman, "Decisions about dendritic cells: past, present, and future," *Annual Review of Immunology*, vol. 30, pp. 1–22, 2012.

[4] C. Ohnmacht, A. Pullner, S. B. S. King et al., "Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity," *Journal of Experimental Medicine*, vol. 206, no. 3, pp. 549–559, 2009.

[5] K. J. Maloy and F. Powrie, "Intestinal homeostasis and its breakdown in inflammatory bowel disease," *Nature*, vol. 474, no. 7351, pp. 298–306, 2011.

[6] S. Manicassamy and B. Pulendran, "Dendritic cell control of tolerogenic responses," *Immunological Reviews*, vol. 241, no. 1, pp. 206–227, 2011.

[7] M. Gross, T. M. Salame, and S. Jung, "Guardians of the gut—murine intestinal macrophages and dendritic cells," *Frontiers in Immunology*, vol. 6, article 254, 2015.

[8] M. Bogunovic, F. Ginhoux, J. Helft et al., "Origin of the lamina propria dendritic cell network," *Immunity*, vol. 31, no. 3, pp. 513–525, 2009.

[9] J. C. Miller, B. D. Brown, T. Shay et al., "Deciphering the transcriptional network of the dendritic cell lineage," *Nature Immunology*, vol. 13, no. 9, pp. 888–899, 2012.

[10] C. Varol, A. Vallon-Eberhard, E. Elinav et al., "Intestinal lamina propria dendritic cell subsets have different origin and functions," *Immunity*, vol. 31, no. 3, pp. 502–512, 2009.

[11] M. Guilliams, F. Ginhoux, C. Jakubzick et al., "Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny," *Nature Reviews Immunology*, vol. 14, no. 8, pp. 571–578, 2014.

[12] M. Guilliams and L. van de Laar, "A Hitchhiker's guide to myeloid cell subsets: practical implementation of a novel mononuclear phagocyte classification system," *Frontiers in Immunology*, vol. 6, article 406, 2015.

[13] C. L. Scott, C. C. Bain, P. B. Wright et al., "CCR2(+)CD103(−) intestinal dendritic cells develop from DC-committed precursors and induce interleukin-17 production by T cells," *Mucosal Immunology*, vol. 8, pp. 327–339, 2015.

[14] E. L. Gautier, T. Shay, J. Miller et al., "Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages," *Nature Immunology*, vol. 13, no. 11, pp. 1118–1128, 2012.

[15] C. C. Bain, C. L. Scott, H. Uronen-Hansson et al., "Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6Chi monocyte precursors," *Mucosal Immunology*, vol. 6, no. 3, pp. 498–510, 2013.

[16] A. Rivollier, J. He, A. Kole, V. Valatas, and B. L. Kelsall, "Inflammation switches the differentiation program of Ly6chi monocytes from antiinflammatory macrophages to inflammatory dendritic cells in the colon," *Journal of Experimental Medicine*, vol. 209, no. 1, pp. 139–155, 2012.

[17] F. Geissmann, S. Gordon, D. A. Hume, A. M. Mowat, and G. J. Randolph, "Unravelling mononuclear phagocyte heterogeneity," *Nature Reviews Immunology*, vol. 10, no. 6, pp. 453–460, 2010.

[18] D. A. Hume, "Differentiation and heterogeneity in the mononuclear phagocyte system," *Mucosal Immunology*, vol. 1, no. 6, pp. 432–441, 2008.

[19] O. Schulz, E. Jaensson, E. K. Persson et al., "Intestinal CD103$^+$, but not CX3CR1$^+$, antigen sampling cells migrate in lymph and serve classical dendritic cell functions," *The Journal of Experimental Medicine*, vol. 206, no. 13, pp. 3101–3114, 2009.

[20] M. Merad, P. Sathe, J. Helft, J. Miller, and A. Mortha, "The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting," *Annual Review of Immunology*, vol. 31, pp. 563–604, 2013.

[21] N. Onai, K. Kurabayashi, M. Hosoi-Amaike et al., "A clonogenic progenitor with prominent plasmacytoid dendritic cell developmental potential," *Immunity*, vol. 38, no. 5, pp. 943–957, 2013.

[22] G. E. Grajales-Reyes, A. Iwata, J. Albring et al., "Batf3 maintains autoactivation of Irf8 for commitment of a CD8α^+ conventional DC clonogenic progenitor," *Nature Immunology*, vol. 16, pp. 708–717, 2015.

[23] J. T. Jackson, Y. Hu, R. Liu et al., "Id2 expression delineates differential checkpoints in the genetic program of CD8α^+ and CD103$^+$ dendritic cell lineages," *The EMBO Journal*, vol. 30, no. 13, pp. 2690–2704, 2011.

[24] M. Kashiwada, N.-L. L. Pham, L. L. Pewe, J. T. Harty, and P. B. Rothman, "NFIL3/E4BP4 is a key transcription factor for CD8α^+ dendritic cell development," *Blood*, vol. 117, no. 23, pp. 6193–6197, 2011.

[25] B. Cisse, M. L. Caton, M. Lehner et al., "Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development," *Cell*, vol. 135, no. 1, pp. 37–48, 2008.

[26] S. Gurka, E. Hartung, M. Becker et al., "Mouse conventional dendritic cells can be universally classified based on the mutually exclusive expression of XCR1 and SIRPα," *Frontiers in Immunology*, vol. 6, article 35, 2015.

[27] L. Wu, A. D'Amico, K. D. Winkel, M. Suter, D. Lo, and K. Shortman, "RelB is essential for the development of myeloid-related CD8α- dendritic cells but not of lymphoid-related CD8α^+ dendritic cells," *Immunity*, vol. 9, no. 6, pp. 839–847, 1998.

[28] M. L. Caton, M. R. Smith-Raska, and B. Reizis, "Notch-RBP-J signaling controls the homeostasis of CD8$^-$ dendritic cells in the spleen," *The Journal of Experimental Medicine*, vol. 204, no. 7, pp. 1653–1664, 2007.

[29] E. K. Persson, H. Uronen-Hansson, M. Semmrich et al., "IRF4 transcription-factor-dependent CD103$^+$CD11b$^+$ dendritic cells drive mucosal T helper 17 cell differentiation," *Immunity*, vol. 38, no. 5, pp. 958–969, 2013.

[30] B. T. Edelson, K. C. Wumesh, R. Juang et al., "Peripheral CD103$^+$ dendritic cells form a unified subset developmentally related to CD8α^+ conventional dendritic cells," *Journal of Experimental Medicine*, vol. 207, no. 4, pp. 823–836, 2010.

[31] B. Vander Lugt, A. A. Khan, J. A. Hackney et al., "Transcriptional programming of dendritic cells for enhanced MHC class II antigen presentation," *Nature Immunology*, vol. 15, no. 2, pp. 161–167, 2014.

[32] A. Schlitzer, N. McGovern, P. Teo et al., "IRF4 transcription factor-dependent CD11b$^+$ dendritic cells in human and mouse control mucosal IL-17 cytokine responses," *Immunity*, vol. 38, no. 5, pp. 970–983, 2013.

[33] W. W. Agace and E. K. Persson, "How vitamin A metabolizing dendritic cells are generated in the gut mucosa," *Trends in Immunology*, vol. 33, no. 1, pp. 42–48, 2012.

[34] J. A. Hall, J. R. Grainger, S. P. Spencer, and Y. Belkaid, "The role of retinoic acid in tolerance and immunity," *Immunity*, vol. 35, no. 1, pp. 13–22, 2011.

[35] M. Guilliams, K. Crozat, S. Henri et al., "Skin-draining lymph nodes contain dermis-derived CD103$^-$ dendritic cells that constitutively produce retinoic acid and induce Foxp3$^+$ regulatory T cells," *Blood*, vol. 115, no. 10, pp. 1958–1968, 2010.

[36] T. L. Denning, B. A. Norris, O. Medina-Contreras et al., "Functional specializations of intestinal dendritic cell and macrophage subsets that control Th17 and regulatory T cell responses are dependent on the T cell/APC ratio, source of mouse strain, and regional localization," *The Journal of Immunology*, vol. 187, no. 2, pp. 733–747, 2011.

[37] B. M. Janelsins, M. Lu, and S. K. Datta, "Altered inactivation of commensal LPS due to acyloxyacyl hydrolase deficiency in colonic dendritic cells impairs mucosal Th17 immunity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 1, pp. 373–378, 2014.

[38] K. Honda and D. R. Littman, "The microbiome in infectious disease and inflammation," *Annual Review of Immunology*, vol. 30, pp. 759–795, 2012.

[39] 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.

[40] K. Fujimoto, T. Karuppuchamy, N. Takemura et al., "A new subset of CD103$^+$CD8α^+ dendritic cells in the small intestine expresses TLR3, TLR7, and TLR9 and induces Th1 response and CTL activity," *The Journal of Immunology*, vol. 186, no. 11, pp. 6287–6295, 2011.

[41] V. Cerovic, S. A. Houston, C. L. Scott et al., "Intestinal CD103$^-$ dendritic cells migrate in lymph and prime effector T cells," *Mucosal Immunology*, vol. 6, no. 1, pp. 104–113, 2013.

[42] C. L. Scott, T. F. P. Z. Murray, K. S. H. Beckham, G. Douce, and A. M. Mowat, "Signal regulatory protein alpha (SIRPα) regulates the homeostasis of CD103$^+$CD11b$^+$ DCs in the intestinal lamina propria," *European Journal of Immunology*, vol. 44, no. 12, pp. 3658–3668, 2014.

[43] E. K. Persson, C. L. Scott, A. M. Mowat, and W. W. Agace, "Dendritic cell subsets in the intestinal lamina propria: ontogeny and function," *European Journal of Immunology*, vol. 43, no. 12, pp. 3098–3107, 2013.

[44] S. Veenbergen, L. A. van Berkel, M. F. du Pré et al., "Colonic tolerance develops in the iliac lymph nodes and can be established independent of CD103$^+$ dendritic cells," *Mucosal Immunology*, 2015.

[45] N. E. Welty, C. Staley, N. Ghilardi, M. J. Sadowsky, B. Z. Igyártó, and D. H. Kaplan, "Intestinal lamina propria dendritic cells maintain T cell homeostasis but do not affect commensalism," *The Journal of Experimental Medicine*, vol. 210, no. 10, pp. 2011–2024, 2013.

[46] N. S. Wilson, L. J. Young, F. Kupresanin et al., "Normal proportion and expression of maturation markers in migratory dendritic cells in the absence of germs or Toll-like receptor signaling," *Immunology and Cell Biology*, vol. 86, no. 2, pp. 200–205, 2008.

[47] C. Qian, J. Liu, and X. Cao, "Innate signaling in the inflammatory immune disorders," *Cytokine and Growth Factor Reviews*, vol. 25, no. 6, pp. 731–738, 2014.

[48] O. Takeuchi and S. Akira, "Pattern recognition receptors and inflammation," *Cell*, vol. 140, no. 6, pp. 805–820, 2010.

[49] J. Brown, H. Wang, G. N. Hajishengallis, and M. Martin, "TLR-signaling networks: an integration of adaptor molecules, kinases, and cross-talk," *Journal of Dental Research*, vol. 90, no. 4, pp. 417–427, 2011.

[50] J. R. McDole, L. W. Wheeler, K. G. McDonald et al., "Goblet cells deliver luminal antigen to CD103$^+$ dendritic cells in the small intestine," *Nature*, vol. 483, no. 7389, pp. 345–349, 2012.

[51] E. Mazzini, L. Massimiliano, G. Penna, and M. Rescigno, "Oral tolerance can be established via gap junction transfer of fed antigens from CX3CR1$^+$ macrophages to CD103$^+$ dendritic cells," *Immunity*, vol. 40, no. 2, pp. 248–261, 2014.

[52] J. Farache, I. Koren, I. Milo et al., "Luminal bacteria recruit CD103$^+$ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation," *Immunity*, vol. 38, no. 3, pp. 581–595, 2013.

[53] A. Vallon-Eberhard, L. Landsman, N. Yogev, B. Verrier, and S. Jung, "Transepithelial pathogen uptake into the small intestinal lamina propria," *Journal of Immunology*, vol. 176, no. 4, pp. 2465–2469, 2006.

[54] N. Singh, A. Gurav, S. Sivaprakasam et al., "Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis," *Immunity*, vol. 40, no. 1, pp. 128–139, 2014.

[55] D. Han, M. C. Walsh, P. J. Cejas et al., "Dendritic cell expression of the signaling molecule TRAF6 is critical for gut microbiota-dependent immune tolerance," *Immunity*, vol. 38, no. 6, pp. 1211–1222, 2013.

[56] L. Delamarre, H. Holcombe, and I. Mellman, "Presentation of exogenous antigens on major histocompatibility complex (MHC) class I and MHC class II molecules is differentially regulated during dendritic cell maturation," *The Journal of Experimental Medicine*, vol. 198, no. 1, pp. 111–122, 2003.

[57] J. Liu and X. Cao, "Regulatory dendritic cells in autoimmunity: a comprehensive review," *Journal of Autoimmunity*, vol. 63, pp. 1–12, 2015.

[58] M. V. Dhodapkar, R. M. Steinman, J. Krasovsky, C. Munz, and N. Bhardwaj, "Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells," *Journal of Experimental Medicine*, vol. 193, no. 2, pp. 233–238, 2001.

[59] J. S. Frick, N. Zahir, M. Müller et al., "Colitogenic and non-colitogenic commensal bacteria differentially trigger DC maturation and Th cell polarization: an important role for IL-6," *European Journal of Immunology*, vol. 36, no. 6, pp. 1537–1547, 2006.

[60] M. B. Lutz, "Therapeutic potential of semi-mature dendritic cells for tolerance induction," *Frontiers in Immunology*, vol. 3, article 123, 2012.

[61] M. Menges, S. Rößner, C. Voigtländer et al., "Repetitive injections of dendritic cells matured with tumor necrosis factor α induce antigen-specific protection of mice from autoimmunity," *The Journal of Experimental Medicine*, vol. 195, no. 1, pp. 15–21, 2002.

[62] W. W. J. Unger, S. Laban, F. S. Kleijwegt, A. R. Van Der Slik, and B. O. Roep, "Induction of Treg by monocyte-derived DC modulated by vitamin D_3 or dexamethasone: differential role for PD-L1," *European Journal of Immunology*, vol. 39, no. 11, pp. 3147–3159, 2009.

[63] A. B. Addi, A. Lefort, X. Hua et al., "Modulation of murine dendritic cell function by adenine nucleotides and adenosine: involvement of the A_{2B} receptor," *European Journal of Immunology*, vol. 38, no. 6, pp. 1610–1620, 2008.

[64] J. Geisel, F. Kahl, M. Müller et al., "IL-6 and maturation govern TLR2 and TLR4 induced TLR agonist tolerance and cross-tolerance in dendritic cells," *The Journal of Immunology*, vol. 179, no. 9, pp. 5811–5818, 2007.

[65] E. Ozeri, M. Mizrahi, G. Shahaf, and E. C. Lewis, "α-1 antitrypsin promotes semimature, IL-10-producing and readily migrating tolerogenic dendritic cells," *Journal of Immunology*, vol. 189, no. 1, pp. 146–153, 2012.

[66] Y. Shen, M. L. G. Torchia, G. W. Lawson, C. L. Karp, J. D. Ashwell, and S. K. Mazmanian, "Outer membrane vesicles of a human commensal mediate immune regulation and disease protection," *Cell Host and Microbe*, vol. 12, no. 4, pp. 509–520, 2012.

[67] J. K. Nono, K. Pletinckx, M. B. Lutz, and K. Brehm, "Excretory/secretory-products of *Echinococcus multilocularis* larvae induce apoptosis and tolerogenic properties in dendritic cells in vitro," *PLoS Neglected Tropical Diseases*, vol. 6, no. 2, Article ID e1516, 2012.

[68] A. E. Morelli and A. W. Thomson, "Tolerogenic dendritic cells and the quest for transplant tolerance," *Nature Reviews Immunology*, vol. 7, no. 8, pp. 610–621, 2007.

[69] A. Iwasaki and R. Medzhitov, "Control of adaptive immunity by the innate immune system," *Nature Immunology*, vol. 16, no. 4, pp. 343–353, 2015.

[70] E. C. Steinbach and S. E. Plevy, "The role of macrophages and dendritic cells in the initiation of inflammation in IBD," *Inflammatory Bowel Diseases*, vol. 20, no. 1, pp. 166–175, 2014.

[71] R. B. Sartor, "Microbial influences in inflammatory bowel diseases," *Gastroenterology*, vol. 134, no. 2, pp. 577–594, 2008.

[72] J. L. Coombes and F. Powrie, "Dendritic cells in intestinal immune regulation," *Nature Reviews Immunology*, vol. 8, no. 6, pp. 435–446, 2008.

[73] S. Rakoff-Nahoum, L. Hao, and R. Medzhitov, "Role of toll-like receptors in spontaneous commensal-dependent colitis," *Immunity*, vol. 25, no. 2, pp. 319–329, 2006.

[74] J. M. Kim, J. P. Rasmussen, and A. Y. Rudensky, "Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice," *Nature Immunology*, vol. 8, no. 2, pp. 191–197, 2007.

[75] D. Haribhai, J. B. Williams, S. Jia et al., "A requisite role for induced regulatory T cells in tolerance based on expanding antigen receptor diversity," *Immunity*, vol. 35, no. 1, pp. 109–122, 2011.

[76] M. Kronenberg and A. Rudensky, "Regulation of immunity by self-reactive T cells," *Nature*, vol. 435, no. 7042, pp. 598–604, 2005.

[77] Q. Tang and J. A. Bluestone, "The $Foxp3^+$ regulatory T cell: a jack of all trades, master of regulation," *Nature Immunology*, vol. 9, no. 3, pp. 239–244, 2008.

[78] J. L. Coombes, K. R. R. Siddiqui, C. V. Arancibia-Cárcamo et al., "A functionally specialized population of mucosal $CD103^+$ DCs induces $Foxp3^+$ regulatory T cells via a TGF-β and retinoic acid-dependent mechanism," *Journal of Experimental Medicine*, vol. 204, no. 8, pp. 1757–1764, 2007.

[79] N. Toledano, D. Gur-Wahnon, A. Ben-Yehuda, and J. Rachmilewitz, "Novel CD47: SIRPα dependent mechanism for the activation of STAT3 in antigen-presenting cell," *PLoS ONE*, vol. 8, no. 9, Article ID e75595, 2013.

[80] F. Cheng, H.-W. Wang, A. Cuenca et al., "A critical role for Stat3 signaling in immune tolerance," *Immunity*, vol. 19, no. 3, pp. 425–436, 2003.

[81] V. Seiffart, J. Zoeller, R. Klopfleisch et al., "IL10-deficiency in $CD4^+$ T cells exacerbates the IFNγ and IL17 response during bacteria induced colitis," *Cellular Physiology and Biochemistry*, vol. 36, no. 4, pp. 1259–1273, 2015.

[82] M. Saraiva and A. O'Garra, "The regulation of IL-10 production by immune cells," *Nature Reviews Immunology*, vol. 10, no. 3, pp. 170–181, 2010.

[83] D. S. Shouval, J. Ouahed, A. Biswas et al., "Interleukin 10 receptor signaling: master regulator of intestinal mucosal homeostasis in mice and humans," *Advances in Immunology*, vol. 122, pp. 177–210, 2014.

[84] B. Begue, J. Verdier, F. Rieux-Laucat et al., "Defective IL10 signaling defining a subgroup of patients with inflammatory bowel disease," *American Journal of Gastroenterology*, vol. 106, no. 8, pp. 1544–1555, 2011.

[85] F. E. Buruiana, I. Sola, and P. Alonso-Coello, "Recombinant human interleukin 10 for induction of remission in Crohn's disease," *Cochrane Database of Systematic Reviews*, vol. 11, Article ID CD005109, 2010.

[86] J. Chang, S. L. Kunkel, and C.-H. Chang, "Negative regulation of MyD88-dependent signaling by IL-10 in dendritic cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 43, pp. 18327–18332, 2009.

[87] T. Kawai and S. Akira, "TLR signaling," *Cell Death and Differentiation*, vol. 13, no. 5, pp. 816–825, 2006.

[88] M. Rescigno and A. Di Sabatino, "Dendritic cells in intestinal homeostasis and disease," *Journal of Clinical Investigation*, vol. 119, no. 9, pp. 2441–2450, 2009.

[89] C. Porta, M. Rimoldi, G. Raes et al., "Tolerance and M2 (alternative) macrophage polarization are related processes orchestrated by p50 nuclear factor κB," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 35, pp. 14978–14983, 2009.

[90] G. Bonizzi and M. Karin, "The two NF-κB activation pathways and their role in innate and adaptive immunity," *Trends in Immunology*, vol. 25, no. 6, pp. 280–288, 2004.

[91] P. Larghi, C. Porta, E. Riboldi et al., "The p50 subunit of NF-κB orchestrates dendritic cell lifespan and activation of adaptive immunity," *PLoS ONE*, vol. 7, no. 9, Article ID e45279, 2012.

[92] E. M. Shevach, "Mechanisms of $foxp3^+$ T regulatory cell-mediated suppression," *Immunity*, vol. 30, no. 5, pp. 636–645, 2009.

[93] H. W. L. Ziegler-Heitbrock, I. Petersmann, and M. Frankenberger, "p50 (NF-κB1) is upregulated in LPS tolerant P388D1 murine macrophages," *Immunobiology*, vol. 198, no. 1–3, pp. 73–80, 1997.

[94] S. Manicassamy, B. Reizis, R. Ravindran et al., "Activation of β-catenin in dendritic cells regulates immunity versus tolerance in the intestine," *Science*, vol. 329, no. 5993, pp. 849–853, 2010.

[95] R. Liberman, S. Bond, M. G. Shainheit, M. J. Stadecker, and M. Forgac, "Regulated assembly of vacuolar ATPase is increased during cluster disruption-induced maturation of dendritic cells through a phosphatidylinositol 3-Kinase/mTOR-dependent pathway," *The Journal of Biological Chemistry*, vol. 289, no. 3, pp. 1355–1363, 2014.

[96] E. S. Trombetta, M. Ebersold, W. Garrett, M. Pypaert, and I. Mellman, "Activation of lysosomal function during dendritic cell maturation," *Science*, vol. 299, no. 5611, pp. 1400–1403, 2003.

[97] B. Vander Lugt, Z. T. Beck, R. C. Fuhlbrigge, N. Hacohen, J. J. Campbell, and M. Boes, "TGF-β suppresses β-catenin-dependent tolerogenic activation program in dendritic cells," *PLoS ONE*, vol. 6, no. 5, Article ID e20099, 2011.

[98] G. Huang, L. Z. Shi, and H. Chi, "Regulation of JNK and p38 MAPK in the immune system: signal integration, propagation and termination," *Cytokine*, vol. 48, no. 3, pp. 161–169, 2009.

[99] G. Huang, Y. Wang, P. Vogel, T.-D. Kanneganti, K. Otsu, and H. Chi, "Signaling via the kinase p38α programs dendritic cells to drive T_H17 differentiation and autoimmune inflammation," *Nature Immunology*, vol. 13, no. 2, pp. 152–161, 2012.

[100] J. Ochoa-Repáraz, D. W. Mielcarz, Y. Wang et al., "A polysaccharide from the human commensal *Bacteroides fragilis* protects against CNS demyelinating disease," *Mucosal Immunology*, vol. 3, no. 5, pp. 487–495, 2010.

[101] J. Ochoa-Repáraz, D. W. Mielcarz, L. E. Ditrio et al., "Central nervous system demyelinating disease protection by the human commensal *Bacteroides fragilis* depends on polysaccharide A expression," *Journal of Immunology*, vol. 185, no. 7, pp. 4101–4108, 2010.

[102] J. L. Round, S. M. Lee, J. Li et al., "The toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota," *Science*, vol. 332, no. 6032, pp. 974–977, 2011.

[103] J. M. Salvador, P. R. Mittelstadt, G. I. Belova, A. J. Fornace Jr., and J. D. Ashwell, "The autoimmune suppressor Gadd45α inhibits the T cell alternative p38 activation pathway," *Nature Immunology*, vol. 6, no. 4, pp. 396–402, 2005.

[104] M. Waidmann, O. Bechtold, J.-S. Frick et al., "Bacteroides vulgatus protects against *Escherichia coli*-induced colitis in gnotobiotic interleukin-2-deficient mice," *Gastroenterology*, vol. 125, no. 1, pp. 162–177, 2003.

[105] M. Müller, K. Fink, J. Geisel et al., "Intestinal colonization of IL-2 deficient mice with non-colitogenic *B. vulgatus* prevents DC maturation and T-cell polarization," *PLoS ONE*, vol. 3, no. 6, Article ID e2376, 2008.

[106] A.-M. Gerlach, A. Steimle, L. Krampen et al., "Role of CD40 ligation in dendritic cell semimaturation," *BMC Immunology*, vol. 13, article 22, 2012.

[107] K. Gronbach, I. Flade, O. Holst et al., "Endotoxicity of lipopolysaccharide as a determinant of T-cell-mediated colitis induction in mice," *Gastroenterology*, vol. 146, no. 3, pp. 765–775, 2014.

[108] S. Perruche, P. Zhang, Y. Liu, P. Saas, J. A. Bluestone, and W. Chen, "CD3-specific antibody-induced immune tolerance involves transforming growth factor-β from phagocytes digesting apoptotic T cells," *Nature Medicine*, vol. 14, no. 5, pp. 528–535, 2008.

[109] R. Kuang, S. Perruche, and W. Chen, "Apoptotic cell-linked immunoregulation: implications for promoting immune tolerance in transplantation," *Cell & Bioscience*, vol. 5, article 27, 2015.

[110] T. B. da Costa, L. R. Sardinha, R. Larocca, J. P. S. Peron, and L. V. Rizzo, "Allogeneic apoptotic thymocyte-stimulated dendritic cells expand functional regulatory T cells," *Immunology*, vol. 133, no. 1, pp. 123–132, 2011.

[111] M. A. Gleisner, M. Rosemblatt, J. A. Fierro, and M. R. Bono, "Delivery of alloantigens via apoptotic cells generates dendritic cells with an immature tolerogenic phenotype," *Transplantation Proceedings*, vol. 43, no. 6, pp. 2325–2333, 2011.

[112] F. Zhou, E. Lauretti, A. di Meco et al., "Intravenous transfer of apoptotic cell-treated dendritic cells leads to immune tolerance by blocking Th17 cell activity," *Immunobiology*, vol. 218, no. 8, pp. 1069–1076, 2013.

[113] M. J. Perone, S. Bertera, Z. S. Tawadrous et al., "Dendritic cells expressing transgenic galectin-1 delay onset of autoimmune diabetes in mice," *The Journal of Immunology*, vol. 177, no. 8, pp. 5278–5289, 2006.

[114] J. M. Ilarregui, D. O. Croci, G. A. Bianco et al., "Tolerogenic signals delivered by dendritic cells to T cells through a galectin-1-driven immunoregulatory circuit involving interleukin 27 and interleukin 10," *Nature Immunology*, vol. 10, no. 9, pp. 981–991, 2009.

[115] M. Xiang, J. Lu, C. Zhang et al., "Identification and quantification of total coumarins from Urtica dentata hand and its roles in promoting immune tolerance via TLR4-mediated dendritic cell immaturation," *Bioscience, Biotechnology and Biochemistry*, vol. 77, no. 6, pp. 1200–1206, 2013.

[116] H. R. Turnquist, G. Raimondi, A. F. Zahorchak, R. T. Fischer, Z. Wang, and A. W. Thomson, "Rapamycin-conditioned dendritic cells are poor stimulators of allogeneic $CD4^+$ T cells, but enrich for antigen-specific $Foxp3^+$ T regulatory cells and promote organ transplant tolerance," *The Journal of Immunology*, vol. 178, no. 11, pp. 7018–7031, 2007.

[117] S. Marin-Gallen, X. Clemente-Casares, R. Planas et al., "Dendritic cells pulsed with antigen-specific apoptotic bodies prevent experimental type 1 diabetes," *Clinical and Experimental Immunology*, vol. 160, no. 2, pp. 207–214, 2010.

[118] M. Knip and H. Siljander, "Autoimmune mechanisms in type 1 diabetes," *Autoimmunity Reviews*, vol. 7, no. 7, pp. 550–557, 2008.

[119] R. Cabezón, E. Ricart, C. España, J. Panés, and D. Benitez-Ribas, "Gram-negative enterobacteria induce tolerogenic maturation in dexamethasone conditioned dendritic cells," *PLoS ONE*, vol. 7, no. 12, Article ID e52456, 2012.

[120] S. Uematsu, K. Fujimoto, M. H. Jang et al., "Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5," *Nature Immunology*, vol. 9, no. 7, pp. 769–776, 2008.

[121] 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.

Hindawi Publishing Corporation
Journal of Immunology Research
Volume 2016, Article ID 5134329, 8 pages
http://dx.doi.org/10.1155/2016/5134329

Review Article

Dendritic Cells under Hypoxia: How Oxygen Shortage Affects the Linkage between Innate and Adaptive Immunity

Sandra Winning and Joachim Fandrey

Institut für Physiologie, Universität Duisburg-Essen, Hufelandstrasse 55, 45122 Essen, Germany

Correspondence should be addressed to Joachim Fandrey; joachim.fandrey@uni-due.de

Received 9 November 2015; Revised 7 January 2016; Accepted 13 January 2016

Academic Editor: Silvia Beatriz Boscardin

Dendritic cells (DCs) are considered as one of the main regulators of immune responses. They collect antigens, process them, and present typical antigenic structures to lymphocytes, thereby inducing an adaptive immune response. All these processes take place under conditions of oxygen shortage (hypoxia) which is often not considered in experimental settings. This review highlights how deeply hypoxia modulates human as well as mouse immature and mature dendritic cell functions. It tries to link *in vitro* results to actual *in vivo* studies and outlines how hypoxia-mediated shaping of dendritic cells affects the activation of (innate) immunity.

1. Introduction

Dendritic cells (DCs) are major directors of immune responses [1]. DCs are part of the innate immune system and function as sentinels for pathogens at potential sites of invasion (e.g., the skin or the gastrointestinal tract). Once they have recognized pathogens DCs capture them and process the respective antigen structures. Proteins are then converted into peptides which are subsequently presented on major histocompatibility complex (MHC) molecules and recognized by T lymphocytes [2]. The dendritic cell drives the immune response depending on the kind of antigen it has taken up. If the host needs defense against an invaded pathogen, DCs present the antigenic peptides to cytotoxic $CD8^+$ T cells and proinflammatory $CD4^+$ T helper (Th1) cells activating the lymphocytes via direct cell-cell contact and proinflammatory cytokines such as interleukin- (IL-) 12 or IL-6 [3, 4]. If autoimmunity or abundant inflammation needs to be dampened DCs interact with regulatory T cells (Treg) directly and via secretion of anti-inflammatory cytokines such as IL-10 or retinoic acid (RA) [5–7]. What is often hardly considered in experimental settings is the fact that all processes directing the immune response of our organism take place under deprivation of nutrients and oxygen. The interplay of dendritic cells and lymphocytes either takes place in severely inflamed tissue or in secondary lymphoid organs. These tissues have been described to exhibit low oxygen tensions. Oxygen distribution in the spleen and lymph nodes is highly variable and hypoxic lymphocytes have been identified in both organs [8]. The adaptation of cells to reduced oxygen tensions is largely coordinated by hypoxia-inducible factors (HIFs) which have come into the focus of immunological research during the last ten years.

2. Criteria for This Review

Inflammatory hypoxia is increasingly recognized as a critical determinant for the immune response. This review focuses on mouse and human dendritic cells and their maturation and activation under hypoxic conditions. By covering publications on *in vitro* studies a particular emphasis is put on HIFs as coordinators of the genetic response to hypoxia. The availability of mice with a DC-specific HIF-1α k.-o. now allows a first appreciation of the potential role of hypoxia in DC function *in vivo*.

2.1. Hypoxia-Inducible Factors

2.1.1. Posttranslational Modifications. Hypoxia-inducible factors belong to the family of basic helix-loop-helix proteins [9]. They consist of one α- (HIF-1α, HIF-2α, or HIF-3α) and one common β-subunit (HIF-1β or ARNT, aryl carbon

nuclear translocator) forming the DNA-binding transcription factor dimer. Whereas the HIF-1β protein is not affected by changes in oxygen tension HIF-α proteins are only detectable under hypoxic conditions. HIF-1α mRNA is expressed and transcribed in all nucleated cells whereas the expression of HIF-2α is more limited. HIF-2α is found especially in endothelial cells but also in immune cells such as macrophages or dendritic cells. The role of HIF-3α remains poorly defined so far, particularly in immune cells, and this review will therefore focus on HIF-1α and HIF-2α. HIF-α proteins are under tight posttranslational control by oxygen. Under normoxia, specific prolyl hydroxylase domain containing enzymes (PHD 1, 2, and 3) use molecular oxygen to immediately hydroxylate distinct prolyl residues of newly synthesized HIF-α-subunits. Hydroxylated HIF-α (OH-HIF-α) interacts with the von-Hippel-Lindau protein (pVHL) E3 ligase complex that polyubiquinates OH-HIF-α leading to instantaneous proteasomal degradation under normoxia [10]. Thus, HIF-α proteins are oxygen-labile. In contrast, under hypoxia, prolyl hydroxylases lack oxygen as a crucial cosubstrate and are reduced in their enzymatic activity. HIF-αs can accumulate, translocate into the nucleus, and dimerize with HIF-1β. The transcription factor complexes HIF-1 (HIF-1α/HIF-1β) and HIF-2 (HIF-2α/HIF-1β) recruit cofactors such as p300/CBP (cAMP-response element binding protein) and bind to hypoxia-responsive elements of target gene DNA. In addition to proline hydroxylation, HIF-1α and HIF-2α can be hydroxylated at an asparagine residue in their C-terminal part. Asparagine hydroxylation is controlled by an oxygen-sensitive asparagyl hydroxylase, termed factor-inhibiting HIF- (FIH) 1. FIH-1 activity under normoxia prevents cofactor recruitment and transcriptional activity of the HIF complex. HIFs have been shown to regulate more than 100 genes that are involved in glucose metabolism, cell death, cell cycle, angiogenesis, and erythropoiesis [11].

2.1.2. Inflammatory Stimulation. During the last decade it has been recognized that many other factors are able to induce HIF-α although HIF-1α has been studied more thoroughly than HIF-2α. Bacterial lipopolysaccharides (LPS) are able to induce the NF-κB pathway and HIF-1α mRNA has been shown to be a target of classical NF-κB activation by several groups [12–15]. Recently it was shown that HIF-1 is one of the essential modulators of the cytokine response to bacterial LPS as it is crucial for the synthesis of IL-1β [16]. LPS induce intracellular succinate which stabilizes HIF-α protein via PHD inhibition. In macrophages, this led to increased IL-1β synthesis and release [16]. Furthermore, HIF-1α protein and mRNA expression have been shown to be induced in macrophages purified from wounds. The authors could show that elevated protein levels hereby depended on the inflammatory cytokine TNF-α [17]. Reactive oxygen species (ROS) have been shown to induce HIF-1α as well, although this effect seemed to be time-dependent as external H_2O_2 induced HIF-1α protein in human osteosarcoma cells at early time points but suppressed it later on. HIF-1 target gene expression was suppressed over the whole time period [18]. Nitric oxide ($NO^{\bullet}$) has been shown to induce HIF-1α in normoxic macrophages stimulating macrophage migration by modulating the actin cytoskeleton via small GTPases [19]. The role of HIF-2 in the function of immune cells has been studied much less. Imtiyaz et al. [20] have characterized macrophages lacking HIF-2α under immunological settings. Macrophage $NO^{\bullet}$ production and expression of costimulatory molecules CD86 and MHCII were unaffected by loss of HIF-2α, but Imtiyaz and coworkers found profound changes in cytokine mRNA expression and protein release after stimulation with LPS and interferon γ (IFNγ). Murine bone-marrow-derived macrophages lacking HIF-2α showed reduced induction of IL-1β, IL12p35, Cxcl2, and IL-6 mRNA levels under inflammatory hypoxia whereas hypoxia alone if at all only moderately affected mRNA expression of these genes. Moving to *in vivo* models the authors found that macrophage HIF-2α was required for an adequate immune response to cutaneous and peritoneal irritants [20]. Furthermore, loss of macrophage HIF-2α prevented infiltration of tumor associated macrophages (TAMs) in models of hepatocellular carcinoma and colitis-associated cancer which inhibited tumor growth [20]. The role of HIF-2 in the function of dendritic cells under hypoxia is almost completely unexplored, although Filippi et al. could show that human dendritic cells express HIF-2α mRNA [21].

HIF-1 activity has been shown to be induced by a number of viral infections as well. The human immunodeficiency virus HIV-1 induces HIF-1α protein via induction of intracellular ROS. HIF-1 associates with the HIV long-term repeat to induce HIV gene transcription [22]. Hepatitis Bx protein (Hbx) increases the stability of HIF-1α protein via p42/44 mitogen-activated protein kinases (MAPK). Transactivation of HIF-1 is also increased as Hbx induces the interaction of the transcription factor with CBP, one of the cofactors HIFs recruit for DNA binding [23]. Kaposi's sarcoma-associated herpesvirus (KSHV) and Epstein-Barr-Virus (EBV) induce HIF-1α protein by increasing the proteasomal degradation of proteins involved in the normoxic degradation of HIF-1α. LANA, KSHV latency-associated nuclear antigen [24], increases the degradation of pVHL whereas the PHDs 1 and 3 are degraded by proteasomes upon EBV infection [25]. Furthermore, KSHV expresses functional HREs in the promoter regions of viral genes which could be activated *in vitro* by binding of either HIF-1α or HIF-2α [26]. Taking these data into consideration, activation of HIF-1 (and potentially HIF-2) in viral infections seems to bring benefit to the pathogen rather than the host.

2.2. Dendritic Cells and HIFs. DCs are central in coordinating immune responses against pathogens: whenever they register pathogens they pick them up, process the proteins of pathogens, and present typical antigenic peptides to cells of the adaptive immunity. These processes do not only activate the cell but also induce DC differentiation and maturation and make them migrate towards secondary lymphoid organs. DC differentiation and maturation are accompanied and at least in part defined by the upregulation of costimulatory molecules such as CD80 and CD86 [27]. Furthermore, mature dendritic cells express high levels of surface MHCII and CD40 and are able to secrete IL-12 [27]. This is important as DCs have to function as antigen presenters and producers

of IL-12 at the same time to induce differentiation and proliferation of Th1 cells [3]. Until now, several groups have analyzed DC function under hypoxic conditions.

2.3. In Vitro Analysis. For human DCs it has been shown that differentiation of DCs from blood monocytes under hypoxia resulted in a more active phenotype exhibiting a higher ability to stimulate allogeneic T cell responses. Furthermore, hypoxic immature DCs have been shown to downregulate bacterial phagocytosis and exhibit an increased migratory capacity. Several groups have contributed to the current knowledge on the underlying mechanisms: Elia et al. [28] and Ricciardi et al. [29] have found higher expression of the costimulatory molecules CD80, HLA class II, and CD86 on the cellular surface of hypoxic immature DCs. In contrast, costimulatory molecule expression appeared not to be different between mature hypoxic or normoxic DCs [28]. Ogino et al. [30] reported that hypoxic immature like mature DC exhibited higher allo-T cell stimulation than normoxic DCs. This finding was partly supported by Elia et al. [28] who also found this effect for hypoxic immature DCs. Spirig et al. [31] exposed immature monocyte-derived DCs to hypoxia but could not detect any differences in the expression of costimulatory markers. In turn, LPS-mediated maturation of these cells was augmented by hypoxic conditions [31]. Rama et al. [32] in contrast observed a hypoxia-mediated differentiation of immature human monocyte-derived DCs. They exposed the cells for longer time periods to harsher hypoxic conditions than Spirig and coworkers and could detect a higher expression of CD40 after hypoxic treatment. Hypoxia also led to higher T cell stimulatory activity of DCs and this effect could be blocked by inhibition of the HIF pathway [32]. Of note, maturation of DCs under hypoxia leads to a shift in the expression of chemokines and chemokine receptors. While chemokines get downregulated chemokine receptors are upregulated indicating that hypoxia may favor migratory capacity of mature DCs carrying cytokine receptors rather than immunologic functions such as immune cell recruitment [29, 33]. Filippi et al. could recently show that short-term hypoxia induces the migratory capacity of immature and mature DCs in an *in vitro* migration assay and that this effect depends on the expression of HIF-1α [21]. In contrast to the enhanced migratory capacity hypoxic DCs exhibit a reduced phagocytic activity. Several groups have shown that hypoxia downregulated antigen uptake by immature DCs [28, 30] in a manner that seemed to be independent of HIF-1α [30]. These in part divergent reports may in fact be explained by some still existing uncertainties about the actual degree of cellular hypoxia under different experimental conditions. In addition, the duration of hypoxic exposure will affect the DC response as well as continuous versus intermittent hypoxia.

Hypoxia did not change costimulatory molecule expression of mature DCs, but several groups have found other hypoxia-dependent changes in mature DC function. Chronic hypoxia promoted the onset of a highly proinflammatory gene expression profile in mature DCs generated from human monocytes [34]. Hypoxic mature DCs thereby showed induced gene expression of cytokines and chemokines that are known to induce endothelial cell survival, recruitment and adhesion of mononuclear phagocytes, and recruitment and activation of predominantly Th1/Th17 cells [34]. Yang et al. [35] extended this knowledge and showed that hypoxic mature DCs upregulated the expression of A2B adenosine receptor (A2BAR) and thereby predominantly induced Th2 activation. In 2011, Bosco et al. found that chronic hypoxia potently induced the cell surface expression of triggering receptor expressed on myeloid cells- (TREM-) 1 on mature DCs [36]. The authors illustrated a transient induction depending on the severity of hypoxia and they identified an HRE in the promoter region of TREM-1. Silencing of HIF-1α decreased TREM-1 protein levels. Additional work of the same group has shown that hypoxia induces TREM-1 expression also in immature DCs [37]. TREM-1 cross-linking has been associated with an induced release of inflammatory cytokines such as TNF-α, IL-6, and chemokines such as CXCL8, CCL4, and CCL5. In addition, TREM-1 cross-linking has been shown to induce the release of IL12p70, a cytokine inducing Th1 immune responses. This would mean that hypoxia does not only favor migration of mature DCs but also modulates their inflammatory repertoire to attract other immune cells and to direct T cell activation. Thus, for human dendritic cells it seems appropriate when Bosco and Varesio claim a "dendritic cell reprogramming by hypoxic environment" [38]. Examples of how hypoxia affects differentiation and cytokine response of human dendritic cells can be found in Table 1.

Jantsch et al. reported that murine bone-marrow-derived dendritic cells upregulate CD80 and CD86 after exposure to hypoxia and bacterial LPS in an HIF-1-dependent manner. Consistent with these findings DCs cultivated under hypoxic conditions were less efficient in antigen uptake as they showed a more mature phenotype. In contrast, hypoxic treatment (24 h) alone was not able to induce expression of costimulatory molecules on the surface of BmDCs [41]. These findings appear to be in conflict with the results of Köhler et al. This group used a conditional knockdown of HIF-1α in BmDCs (by crossing HIF-1$\alpha^{+f/+f}$ mice with CD11c-cre$^{wt/tg}$ animals) rather than a siRNA approach and they kept BmDCs under normoxic or hypoxic conditions during the whole process of differentiation from bone marrow cells [42]. DCs differentiated for six days under hypoxic conditions showed a marked upregulation of CD80, CD86, and MHCII but none of these molecules showed an HIF-1α dependent regulation. These somehow contradictory results may be explained by the different exposure times to hypoxia. The work of Köhler et al. instead revealed changes in cytokine expression of hypoxic DCs which were mainly unaffected by loss of HIF-1α. Only secretion of IL-22 seemed to be HIF-1α dependent when BmDCs were differentiated under hypoxic conditions. In addition, only DCs expressing functional HIF-1α showed an increase of surface CCR7 after hypoxic differentiation. This led to a reduced migration of HIF-1α deficient dendritic cells [42]. Further experiments regarding DC stimulation with defined pathogens revealed that bacterial CpGs but not viral poly(I:C) were able to stabilize HIF-1α protein [43]. Jantsch et al. concluded that MyD88 is essential to induce inflammatory, HIF-1-dependent gene transcription (MyD88 is not involved in intracellular TLR3 signaling

Table 1: Gene expression changes induced by hypoxia in human DCs.

Gene(s)	Changes in gene expression	Gene function	HIF dependency	References
cd80, *cd83*, *cd86*, *hla II*, and *cd40*	↑	Costimulatory molecules	cd83: yes [30] cd40: yes [32]	[28–30, 32]
cx3cr1, *ccr3*, *ccr2*, and *cxcr4*	↑	Chemokine receptors		[29]
ccl13, *ccl14*, *ccl18*, *ccl23*, *ccl24*, and *ccl26*	↓	Chemokines		[29]
tnfsf14	↑	Stimulation of T cells		[29]
vegf	↑	Angiogenesis	Yes	[29]
il-8, *mif*	↑	Inflammatory cytokines	il-8: dependency shown for human mesenchymal stem cells [39] mif: yes [40]	[29]
trem-1	↑	IRS receptor, triggers release of inflammatory cytokines	Yes	[36, 37]
cxcl2, *cxcl3*, *cxcl5*, *cxcl6*, and *cxcl8*	↑	Neutrophil recruitment	HRE found in cxcl2, cxcl5, and cxcl6	[34]
ccl20, *ccl3*, and *ccl5*	↑	Recruitment of activated T cells, monocytes, and immature DCs	HRE found in all of them	[34]
ccl18, *ccl23*	↓	Chemoattractants for naïve/resting T cells	Most likely indirect	[34]
a2bar	↑	Adenosine receptor	Yes	[35]

Table 2: Gene/protein expression changes induced by hypoxia in murine DCs.

Gene(s)/protein(s)	Changes in gene/protein expression	Gene/protein function	HIF dependency	References
cd80, *cd86*	Unaffected in differentiated BmDCs after 24 h of hypoxia ↑ after hypoxia + LPS ↑ after hypoxic differentiation of BmDCs	Costimulatory molecules	Yes No	[41] [42]
nos2	↑ after hypoxia + LPS	ROS production	Yes	[43]
IFNα4, IFNα12	↑ after hypoxia + LPS	Type I interferon	Most likely indirect	[44]
IFN-β	↑ after hypoxia + LPS	Type I interferon	Most likely indirect	[44]
IL-22	↑ after hypoxic differentiation of BmDCs	Inflammatory cytokine	Yes	[42]
CCR7	↑ after hypoxic differentiation of BmDCs	Chemokine receptor	Yes	[42]
id2	↑ after hypoxic differentiation of pDCs	Inhibits pDC lineage determination	Yes	[45]

triggered by poly(I:C)). One potential target gene of HIF-1 in DCs cultivated under inflammatory hypoxia (hypoxia + LPS or hypoxia + CpGs, resp.) was inducible nitric oxide synthase. Along that line Wobben et al. [44] reported that LPS as well as CpGs could stimulate HIF-1α protein in BmDCs generated from control and conditional HIF-1α knockout animals (deletion of the DNA binding domain of HIF-1α under the control of the lyz2-cre-promoter; BmDC cultures of these mice show knockout in generated DCs, although circulating dendritic cells do not express lyz2-cre-promoter). Furthermore, BmDCs lacking functional HIF-1α protein showed severe deficiencies in the release of type I interferons after LPS stimulation and could not induce a proper T cell activation in an *in vitro* $CD8^+$ lymphocyte activation assay [44]. Last but not least HIF-1-induced gene expression has been found to modulate the differentiation of murine plasmacytoid DCs (pDCs). Fms-related tyrosine kinase 3-ligand (flt-3L) induced differentiation of pDCs from murine bone marrow cells was dramatically reduced upon hypoxic cultivation and this effect was not found when pDCs were lacking HIF-1α. Inhibitor of DNA binding 2 (id2) was identified as suppressor of differentiation. id2 thereby seemed to be exclusively regulated by HIF-1 as a loss of HIF-2α in the respective cells could not hinder suppression of differentiation [45]. Examples on how hypoxia affects the differentiation and cytokine response of murine dendritic cells can be found in Table 2.

2.4. In Vivo Models. Until now, there are only few published studies that address the role of HIFs in dendritic cells in inflammatory settings. Weigert et al. [45] have analyzed the role of HIFs for plasmacytoid DC differentiation and found that HIF-1α limited pDC generation in the bone marrow. In its absence, pDC development was encouraged and numbers of pDC increased. When mice with breast tumors in a PyMT-MMTV model were crossed with HIF-$1\alpha^{+f/+f}$ lyz2-cre mice lacking functional HIF-1α in myeloid cells Weigert et al. observed markedly enhanced numbers of pDC within

the tumors compared with WT controls [45]. Whether this affects tumor progression and disease outcome still has to be analyzed. Köhler et al. [42] have assayed the ability of BmDCs differentiated under hypoxic conditions to get recruited to secondary lymphoid organs. For this purpose, they generated differentially labeled BmDCs from HIF-1$\alpha^{+f/+f}$ and HIF-1$\alpha^{+f/+f}$ CD11c-cre mice under normoxic and hypoxic conditions and injected equal amounts of WT and HIF-1α k.-o. BmDCs into the footpad of mice. Due to the different labels they could distinguish how many WT or knockout DCs had migrated towards the popliteal lymph nodes and found that the hypoxic increase in DC migration was HIF-1 dependent [42]. Three other models have been used to elucidate the influence of dendritic cell HIF expression on their interaction with T cells. First, vaccination efficacy has been addressed and second two models of inflammation were investigated, namely, *Leishmania* infection and dextran sodium sulfate- (DSS-) induced murine colitis [46–48]. Bhandari et al. studied HIF-1$\alpha^{+f/+f}$ Tie2-cre mice showing knockout not only in endothelial but also in hematopoietic cells. They vaccinated HIF-1$\alpha^{+f/+f}$ and HIF-1$\alpha^{+f/+f}$ Tie2-cre mice with a synthetic OVA peptide that specifically induced a $CD8^+$ T cell response. Analysis of IFNγ release by specific T cells in the spleens eight days after vaccination revealed that dendritic cells lacking HIF-1α very insufficiently induced T cell activation compared to WT DCs. In addition to this, HIF-1$\alpha^{+f/+f}$ Tie2-cre mice showed reduced titers of specific OVA antibodies [46].

Very recent studies used mouse models with dendritic HIF-1α knockout in inflammatory settings *in vivo*. Hammami et al. [47] analyzed HIF-1$\alpha^{+f/+f}$ and HIF-1$\alpha^{+f/+f}$ CD11c-cre$^{wt/tg}$ animals in *Leishmania* infection. Somehow in contrast to Bhandari et al. [46] they found that ablation of HIF-1α in dendritic cells resulted in a more efficient immune response. This comprised increased production of IL-12, induced expansion of $CD8^+$ cells, and higher frequency of short-lived effector cells, a specialized $CD8^+$ T cell population that can be found in resolving acute infection. *Leishmania* infection led to upregulation of HIF-1α in splenocytes. This could be shown to restrict $CD8^+$ T cell expansion as hemizygous HIF-1$\alpha^{+/-}$ mice exhibited a significantly higher expansion of $CD8^+$ cells. HIF-1α expression in dendritic cells in turn exacerbated disease [47].

Fully in line with these findings that HIF-1α dampens the inflammatory response, but with a different consequence for the outcome of these mice, Flück et al. reported that HIF-1α is essential for dendritic cells to induce regulatory T cells (Tregs) in a model of acute DSS-colitis [48]. HIF-1$\alpha^{+f/+f}$ mice and HIF-1$\alpha^{+f/+f}$ CD11c-cre$^{wt/tg}$ animals received equal amounts of DSS, but loss of dendritic HIF-1α caused more abundant inflammation and worse outcome for the animals. Mesenteric lymph nodes of HIF-1$\alpha^{+f/+f}$ CD11c-cre$^{wt/tg}$ animals showed significantly reduced expression of the anti-inflammatory cytokine IL-10 and of transforming growth factor- (TGF-) β—both are potent inducers of Tregs. In addition, the authors found a strongly reduced expression of aldehyde dehydrogenase (Aldh) 1a2, which is necessary for dendritic cells to catalyze retinal to retinoic acid (RA). RA also promotes Treg differentiation in the lymph node [7]. T cells of these lymph nodes in turn showed reduced expression of the retinoic acid receptor (RAR) α and diminished expression of the gut homing markers CCR9 and $\alpha 4\beta 7$ integrin. HIF-1α deficiency in DCs thus abrogated their ability to induce Treg proliferation in secondary lymphoid organs and disrupted Treg homing towards the inflamed gut. Apart from the interplay between dendritic cells and T cells predominantly occurring in the mesenteric lymph nodes the authors could also show that the loss of HIF-1α by DCs affected the crosstalk between DCs and intestinal epithelial cells (IECs) causing IECs to produce more mucins and therefore overcome intestinal barrier damage. Another hint that loss of dendritic HIF-1α may affect the crosstalk between DCs and IECs was the finding that only HIF-1$\alpha^{+f/+f}$ mice exhibited increased levels of thymic stromal lymphopoietin receptor (TSLPR) in DSS colitis. IECs release TSLP, which conditions mucosal DCs to noninflammatory tolerogenic DCs. A reduced expression of the respective receptor may drive DCs towards a more inflammatory phenotype. Figure 1 summarizes the effects of (inflammatory) hypoxia and HIF-1α knockout on dendritic cells as it has been discussed above.

3. Conclusion

Dendritic cells normally have to function under conditions of inflammatory hypoxia. Not only inflammatory stimuli, but also hypoxia profoundly affects their cellular function. The key transcription factors regulating DCs' adaptation to conditions of (inflammatory) hypoxia are hypoxia-inducible factors (HIFs) 1 and 2.

Hypoxic culture of immature DCs induces expression of costimulatory molecules such as CD80, CD86, and HLA class II/MHCII [28, 29]. Whether these effects are dependent on hypoxia-inducible factor- (HIF-) 1 or not is still under debate [41, 42]. In contrast, HIF-1 is well recognized to affect migratory capacities of human and murine dendritic cells, most likely by shaping the cellular chemokine/chemokine receptor profile [21, 29]. Until now, only very few *in vivo* studies have addressed the role of HIFs for dendritic cell function; however these studies indicate that HIF-1 affects (i) differentiation of plasmacytoid dendritic cells in the bone marrow [45], (ii) migration of DCs towards secondary lymphoid organs [42], (iii) $CD8^+$ T cell activation and release of pathogen-specific antibodies [46], (iv) expansion of $CD8^+$ T cells and short-lived effector cells [47], and (v) induction of Tregs [48]. The studies of Hammami et al. [47] and Flück et al. [48] used the identical dendritic cell-specific HIF-1α knockout model (HIF-1$\alpha^{+f/+f}$ CD11c-cre$^{wt/tg}$) in different inflammatory settings. Very strikingly, both studies reported findings tending in the same direction but with very different outcomes for the treated animals. In both studies, dendritic cells lacking HIF-1α were the more potent inducers of an inflammatory response—with benefits for the outcome in *Leishmania* infection [47] but abundant inflammation and more severe illness in DSS colitis [48]. Clearly, more *in vivo* studies are needed to better understand the fragile balance between necessary induction of immunity to fight

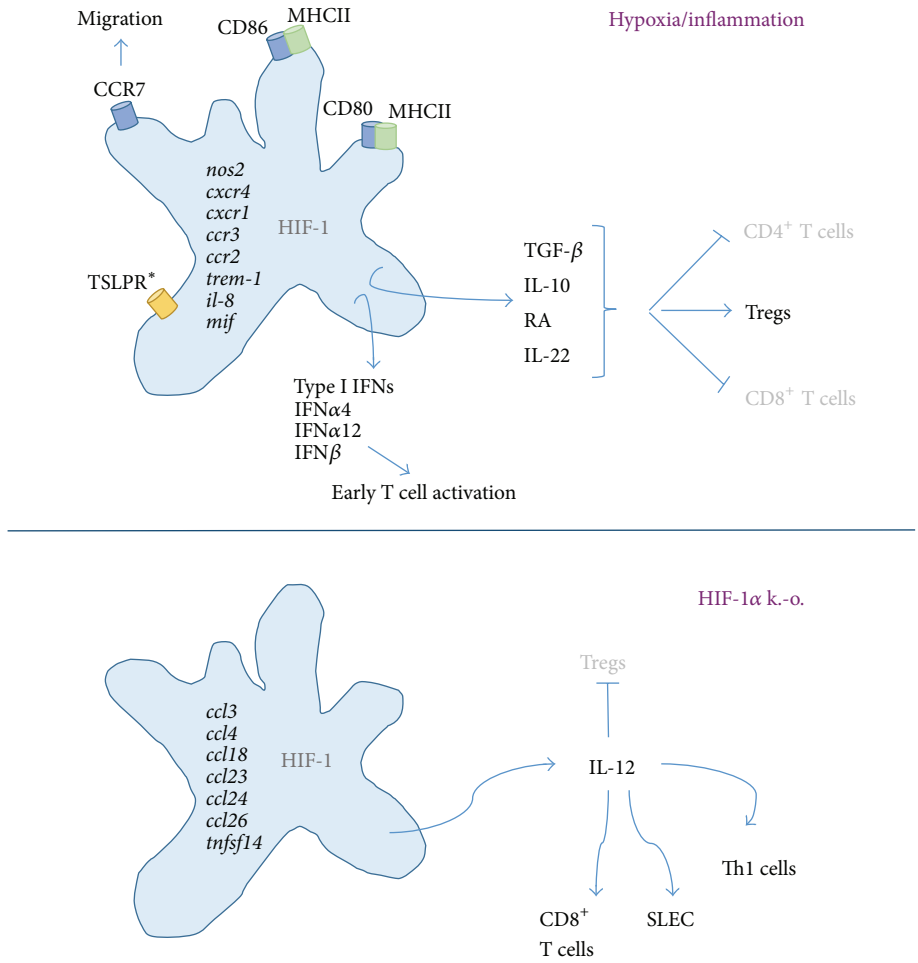


Figure 1: Features of dendritic cells in the presence or absence of active HIF-1α. Dendritic cells expressing HIF-1α have been shown to induce costimulatory molecules (CD80, CD86, and MHCII/HLA class II) under inflammatory hypoxic conditions. Transcripts of *nod2, cxcr4, cxcr1, ccr3, ccr2, trem-1, il-8*, and *mif* are upregulated. Induced expression of CCR7 favors migration of DC towards secondary lymph nodes. These DCs are potent inducers of Tregs via TGF-β, IL-10, RA, and IL-22 although they are able to induce a robust early T cell activation by secretion of type I interferons. Via TSLP, DCs in the gut may be shifted towards a tolerogenic phenotype ($*$: TSLPR expression is limited to gut DCs). Dendritic cells lacking HIF-1α under inflammatory conditions secrete steady high levels of IL-12 and thereby induce a robust activation of proinflammatory T cell populations. They upregulate transcripts of chemokines to attract more immune cells.

a disease and overactivation of immune cells damaging the host. Hypoxia-inducible factors may thereby be important transcriptional regulators in the balance of the immune status of dendritic cells.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] R. M. Steinman, "Decisions about dendritic cells: past, present, and future," *Annual Review of Immunology*, vol. 30, pp. 1–22, 2012.

[2] R. M. Steinman and J. Banchereau, "Taking dendritic cells into medicine," *Nature*, vol. 449, no. 7161, pp. 419–426, 2007.

[3] M. Moser and K. M. Murphy, "Dendritic cell regulation of TH1-TH2 development," *Nature Immunology*, vol. 1, no. 3, pp. 199–205, 2000.

[4] N. Krishnamoorthy, T. Oriss, M. Paglia, A. Ray, and P. Ray, "A critical role for IL-6 secretion by dendritic cells promoting Th2 and limiting Th1 response," *The Journal of Immunology*, vol. 178, p. S181, 2007.

[5] J. M. M. den Haan, G. Kraal, and M. J. Bevan, "Cutting edge: lipopolysaccharide induces IL-10-producing regulatory CD4$^+$ T cells that suppress the CD8$^+$ T cell response," *The Journal of Immunology*, vol. 178, no. 9, pp. 5429–5433, 2007.

[6] K. N. Couper, D. G. Blount, and E. M. Riley, "IL-10: the master regulator of immunity to infection," *Journal of Immunology*, vol. 180, no. 9, pp. 5771–5777, 2008.

[7] M. J. Benson, K. Pino-Lagos, M. Rosemblatt, and R. J. Noelle, "All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation," *The Journal of Experimental Medicine*, vol. 204, no. 8, pp. 1765–1774, 2007.

[8] A. Ohta, R. Diwanji, R. Kini, M. Subramanian, A. Ohta, and M. Sitkovsky, "In vivo T cell activation in lymphoid tissues is inhibited in the oxygen-poor microenvironment," *Frontiers in Immunology*, vol. 2, article 27, 2011.

[9] G. L. Wang, B.-H. Jiang, E. A. Rue, and G. L. Semenza, "Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 12, pp. 5510–5514, 1995.

[10] R. H. Wenger, "Cellular adaptation to hypoxia: O_2-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O_2-regulated gene expression," *The FASEB Journal*, vol. 16, no. 10, pp. 1151–1162, 2002.

[11] V. Nizet and R. S. Johnson, "Interdependence of hypoxic and innate immune responses," *Nature Reviews Immunology*, vol. 9, no. 9, pp. 609–617, 2009.

[12] S. Frede, C. Stockmann, P. Freitag, and J. Fandrey, "Bacterial lipopolysaccharide induces HIF-1 activation in human monocytes via p44/42 MAPK and NF-κB," *Biochemical Journal*, vol. 396, no. 3, pp. 517–527, 2006.

[13] S. Bonello, C. Zähringer, R. S. BelAiba et al., "Reactive oxygen species activate the HIF-1α promoter via a functional NFκB site," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 27, no. 4, pp. 755–761, 2007.

[14] J. Rius, M. Guma, C. Schachtrup et al., "NF-κB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1α," *Nature*, vol. 453, no. 7196, pp. 807–811, 2008.

[15] P. van Uden, N. S. Kenneth, and S. Rocha, "Regulation of hypoxia-inducible factor-1α by NF-κB," *Biochemical Journal*, vol. 412, no. 3, pp. 477–484, 2008.

[16] G. M. Tannahill, A. M. Curtis, J. Adamik et al., "Succinate is an inflammatory signal that induces IL-1β through HIF-1α," *Nature*, vol. 496, no. 7444, pp. 238–242, 2013.

[17] J. E. Albina, B. Mastrofrancesco, J. A. Vessella, C. A. Louis, W. L. Henry Jr., and J. S. Reichner, "HIF-1 expression in healing wounds: HIF-1α induction in primary inflammatory cells by TNF-α," *The American Journal of Physiology—Cell Physiology*, vol. 281, no. 6, pp. C1971–C1977, 2001.

[18] H. Niecknig, S. Tug, B. D. Reyes, M. Kirsch, J. Fandrey, and U. Berchner-Pfannschmidt, "Role of reactive oxygen species in the regulation of HIF-1 by prolyl hydroxylase 2 under mild hypoxia," *Free Radical Research*, vol. 46, no. 6, pp. 705–717, 2012.

[19] J. Zhou, N. Dehne, and B. Brüne, "Nitric oxide causes macrophage migration via the HIF-1-stimulated small GTPases Cdc42 and Rac1," *Free Radical Biology and Medicine*, vol. 47, no. 6, pp. 741–749, 2009.

[20] H. Z. Imtiyaz, E. P. Williams, M. M. Hickey et al., "Hypoxia-inducible factor 2alpha regulates macrophage function in mouse models of acute and tumor inflammation," *The Journal of Clinical Investigation*, vol. 120, no. 8, pp. 2699–2714, 2010.

[21] I. Filippi, E. Morena, C. Aldinucci, F. Carraro, S. Sozzani, and A. Naldini, "Short-Term hypoxia enhances the migratory capability of dendritic cell through HIF-1α and PI3K/Akt pathway," *Journal of Cellular Physiology*, vol. 229, no. 12, pp. 2067–2076, 2014.

[22] S. L. Deshmane, R. Mukerjee, S. Fan et al., "Activation of the oxidative stress pathway by HIV-1 Vpr leads to induction of hypoxia-inducible factor 1α expression," *The Journal of Biological Chemistry*, vol. 284, no. 17, pp. 11364–11373, 2009.

[23] Y.-G. Yoo, S. H. Oh, E. S. Park et al., "Hepatitis B virus X protein enhances transcriptional activity of hypoxia-inducible factor-1α through activation of mitogen-activated protein kinase pathway," *The Journal of Biological Chemistry*, vol. 278, no. 40, pp. 39076–39084, 2003.

[24] Q.-L. Cai, J. S. Knight, S. C. Verma, P. Zald, and E. S. Robertson, "EC5S ubiquitin complex is recruited by KSHV latent antigen LANA for degradation of the VHL and p53 tumor suppressors," *PLoS Pathogens*, vol. 2, no. 10, article e116, 2006.

[25] S. Kondo, S. Y. Seo, T. Yoshizaki et al., "EBV latent membrane protein 1 up-regulates hypoxia-inducible factor 1α through Siah1-mediated down-regulation of prolyl hydroxylases 1 and 3 in nasopharyngeal epithelial cells," *Cancer Research*, vol. 66, no. 20, pp. 9870–9877, 2006.

[26] M. Haque, D. A. Davis, V. Wang, I. Widmer, and R. Yarchoan, "Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) contains hypoxia response elements: relevance to lytic induction by hypoxia," *Journal of Virology*, vol. 77, no. 12, pp. 6761–6768, 2003.

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

[28] A. R. Elia, P. Cappello, M. Puppo et al., "Human dendritic cells differentiated in hypoxia down-modulate antigen uptake and change their chemokine expression profile," *Journal of Leukocyte Biology*, vol. 84, no. 6, pp. 1472–1482, 2008.

[29] A. Ricciardi, A. R. Elia, P. Cappello et al., "Transcriptome of hypoxic immature dendritic cells: modulation of chemokine/receptor expression," *Molecular Cancer Research*, vol. 6, no. 2, pp. 175–185, 2008.

[30] T. Ogino, H. Onishi, H. Suzuki, T. Morisaki, M. Tanaka, and M. Katano, "Inclusive estimation of complex antigen presentation functions of monocyte-derived dendritic cells differentiated under normoxia and hypoxia conditions," *Cancer Immunology, Immunotherapy*, vol. 61, no. 3, pp. 409–424, 2012.

[31] R. Spirig, S. Djafarzadeh, T. Regueira et al., "Effects of TLR agonists on the hypoxia-regulated transcription factor HIF-1α and dendritic cell maturation under normoxic conditions," *PLoS ONE*, vol. 5, no. 6, Article ID e10983, 12 pages, 2010.

[32] I. Rama, B. Bruene, J. Torras et al., "Hypoxia stimulus: an adaptive immune response during dendritic cell maturation," *Kidney International*, vol. 73, no. 7, pp. 816–825, 2008.

[33] M. C. Bosco, M. Puppo, F. Blengio et al., "Monocytes and dendritic cells in a hypoxic environment: spotlights on chemotaxis and migration," *Immunobiology*, vol. 213, no. 9-10, pp. 733–749, 2008.

[34] F. Blengio, F. Raggi, D. Pierobon et al., "The hypoxic environment reprograms the cytokine/chemokine expression profile of human mature dendritic cells," *Immunobiology*, vol. 218, no. 1, pp. 76–89, 2013.

[35] M. Yang, C. Ma, S. Liu et al., "HIF-dependent induction of adenosine receptor A2b skews human dendritic cells to a Th2-stimulating phenotype under hypoxia," *Immunology and Cell Biology*, vol. 88, no. 2, pp. 165–171, 2010.

[36] M. C. Bosco, D. Pierobon, F. Blengio et al., "Hypoxia modulates the gene expression profile of immunoregulatory receptors in human mature dendritic cells: identification of TREM-1 as a novel hypoxic marker in vitro and in vivo," *Blood*, vol. 117, no. 9, pp. 2625–2639, 2011.

[37] D. Pierobon, M. C. Bosco, F. Blengio et al., "Chronic hypoxia reprograms human immature dendritic cells by inducing

a proinflammatory phenotype and TREM-1 expression," *European Journal of Immunology*, vol. 43, no. 4, pp. 949–966, 2013.

[38] M. C. Bosco and L. Varesio, "Dendritic cell reprogramming by the hypoxic environment," *Immunobiology*, vol. 217, no. 12, pp. 1241–1249, 2012.

[39] S. K. Jung, J. H. Kim, H. J. Kim, Y. H. Ji, J. H. Kim, and S. W. Son, "Silver nanoparticle-induced hMSC proliferation is associated with HIF-1α-mediated upregulation of IL-8 expression," *Journal of Investigative Dermatology*, vol. 134, no. 12, pp. 3003–3007, 2014.

[40] G. Zhu, Y. Tang, N. Geng et al., "Hif-α/MIF and NF-κB/IL-6 axes contribute to the recruitment of $CD11b^+Gr\text{-}1^+$ myeloid cells in hypoxic microenvironment of HNSCC," *Neoplasia*, vol. 16, no. 2, pp. 168–179, 2014.

[41] J. Jantsch, D. Chakravortty, N. Turza et al., "Hypoxia and hypoxia-inducible factor-1α modulate lipopolysaccharide-induced dendritic cell activation and function," *The Journal of Immunology*, vol. 180, no. 7, pp. 4697–4705, 2008.

[42] T. Köhler, B. Reizis, R. S. Johnson, H. Weighardt, and I. Förster, "Influence of hypoxia-inducible factor 1α on dendritic cell differentiation and migration," *European Journal of Immunology*, vol. 42, no. 5, pp. 1226–1236, 2012.

[43] J. Jantsch, M. Wiese, J. Schödel et al., "Toll-like receptor activation and hypoxia use distinct signaling pathways to stabilize hypoxia-inducible factor 1α (HIF1A) and result in differential HIF1A-dependent gene expression," *Journal of Leukocyte Biology*, vol. 90, no. 3, pp. 551–562, 2011.

[44] R. Wobben, Y. Hüsecken, C. Lodewick, K. Gibbert, J. Fandrey, and S. Winning, "Role of hypoxia inducible factor-1α for interferon synthesis in mouse dendritic cells," *Biological Chemistry*, vol. 394, no. 4, pp. 495–505, 2013.

[45] A. Weigert, B. Weichand, D. Sekar et al., "HIF-1α is a negative regulator of plasmacytoid DC development in vitro and in vivo," *Blood*, vol. 120, no. 15, pp. 3001–3006, 2012.

[46] T. Bhandari, J. Olson, R. S. Johnson, and V. Nizet, "HIF-1α influences myeloid cell antigen presentation and response to subcutaneous OVA vaccination," *Journal of Molecular Medicine*, vol. 91, no. 10, pp. 1199–1205, 2013.

[47] A. Hammami, T. Charpentier, M. Smans, S. Stäger, and E. Y. Denkers, "IRF-5-mediated inflammation limits CD8+ T cell expansion by inducing HIF-1α and impairing dendritic cell functions during leishmania infection," *PLoS Pathogens*, vol. 11, no. 6, Article ID e1004938, 2015.

[48] K. Flück, G. Breves, J. Fandrey, and S. Winning, "Hypoxia-inducible factor 1 in dendritic cells is crucial for the activation of protective regulatory T cells in murine colitis," *Mucosal Immunology*, 2015.

Hindawi Publishing Corporation
Journal of Immunology Research
Volume 2016, Article ID 3967436, 9 pages
http://dx.doi.org/10.1155/2016/3967436

Review Article

Dendritic Cells and *Leishmania* Infection: Adding Layers of Complexity to a Complex Disease

Daniel Feijó,[1] Rafael Tibúrcio,[1,2] Mariana Ampuero,[1,2] Cláudia Brodskyn,[1,2,3] and Natalia Tavares[1]

[1]*Centro de Pesquisas Gonçalo Moniz (CPqGM), 40296-710 Salvador, BA, Brazil*
[2]*Universidade Federal da Bahia (UFBA), 40170-115 Salvador, BA, Brazil*
[3]*Instituto de Investigação em Imunologia (iii), 01246-903 São Paulo, SP, Brazil*

Correspondence should be addressed to Daniel Feijó; danielffeijo@gmail.com and Natalia Tavares; natalia.tavares@bahia.fiocruz.br

Received 23 October 2015; Accepted 28 December 2015

Academic Editor: Alice O. Kamphorst

Leishmaniasis is a group of neglected diseases whose clinical manifestations depend on factors from the host and the pathogen. It is an important public health problem worldwide caused by the protozoan parasite from the *Leishmania* genus. Cutaneous Leishmaniasis (CL) is the most frequent form of this disease transmitted by the bite of an infected sandfly into the host skin. The parasites can be uptook and/or recognized by macrophages, neutrophils, and/or dendritic cells (DCs). Initially, DCs were described to play a protective role in activating the immune response against *Leishmania* parasites. However, several reports showed a dichotomic role of DCs in modulating the host immune response to susceptibility or resistance in CL. In this review, we discuss (1) the interactions between DCs and parasites from different species of *Leishmania* and (2) the crosstalk of DCs and other cells during CL infection. The complexity of these interactions profoundly affects the adaptive immune response and, consequently, the disease outcome, especially from *Leishmania* species of the New World.

1. Introduction

Leishmaniasis are a complex of vector-borne diseases caused by an intracellular protozoan parasite from *Leishmania* sp. (Kinetoplastida, Trypanosomatidae). Its clinical spectra depends largely on parasite species and host immune response. Although the disease has been known and studied for a long time, it is still considered as a neglected and public health problem worldwide. Such diseases affect approximately 12 million people in 88 countries, where 350 million inhabitants are exposed, mainly in remote rural areas and underserved urban areas [1]. The clinical forms range from asymptomatic infection to two main clinical syndromes: visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL).

VL is a chronic infection, fatal if not treated. It is characterized by progressive fever, weight loss, splenomegaly, hepatomegaly, anemia, and spontaneous bleeding associated with marked inflammatory imbalance [2]. The hallmark of this disease is thought to be a lack of cellular immune response against the parasite and high systemic levels of IFN-g and IL-10 [3].

CL is the most frequent form of this disease. It is characterized by chronic evolution, which affects the skin and cartilaginous structures [4]. The main clinical forms of diseases associated with CL are the Localized Cutaneous Leishmaniasis (LCL), Mucocutaneous Leishmaniasis (ML), disseminated and diffuse Leishmaniasis [1].

LCL is mainly caused by the species *Leishmania tropica*, *L. aethiopica,* and *L. major* in the Old World. However, New World LCL is mainly caused by multiple species of both *Leishmania* subgenera *Leishmania* (*L. amazonensis*, *L. infantum*, *L. mexicana, and L. venezuelensis*) and *Viannia* subgenera (*L. braziliensis*, *L. guyanensis*, *L. panamensis*, and *L. peruviana*). The incubation period lasts on average from 2 weeks to 3 months with the appearance of papules or nodules and, sometimes, is preceded or accompanied by the swelling of underlying nodes. The hallmark of this illness is the development

of single or multiple ulcerated dermal lesions. Over time, the lesion may evolve spontaneously to healing or develop into different frames of gravity in ulceration of the lesion with its expansion [4].

Some patients (a fraction of 3%) may develop the ML, caused by the infection with *L. braziliensis* and *L. guyanensis*. The symptoms are associated with the destruction of the nasal cavity and oropharyngeal tissues [4]. Genetic diversity of *Leishmania* species contributes to the difficulty of controlling the disease and to the increase in the number of cases that are resistant to conventional treatment [5]. Although both forms of CL are rarely fatal, they can cause nasty scars on the skin and severe problems in the oropharyngeal device [4].

Dendritic cells (DCs) are a family of professional antigen-presenting cells (APCs) that resides in all peripheral tissues in an immature state, capable of antigen uptake and processing. As such, they function as sentinel of the immune system. After contact with microorganisms or substances associated with infection or inflammation, DCs undergo a process of maturation and migrate to the T cell areas of lymphoid organs. There, they present antigens to naïve T cells and modulate their responses [6]. The maturation process consists of (1) increased expression of major histocompatibility complex (MHC) and costimulatory molecules, such as CD40, CD80, CD86, and CD54; (2) downregulation of antigen capture and phagocytic capacity; (3) enhanced cytokine secretion; (4) different patterns of chemokine receptor expression and chemokine production, enabling DC migration and recruitment of other cell types [7, 8].

DCs are able to take up antigens via different groups of receptor families, such as Fc receptors, C-type lectin receptors (CLRs), and pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) [9]. The engagement between ligand and its receptor enables DCs to recognize a wide range of microbial stimuli [10].

DCs are a heterogeneous population of cells that can be divided into 2 main categories: the plasmacytoid DCs (pDCs), experts in type I interferon synthesis, and the conventional DCs (cDCs), specialized in antigen capture, processing, and presentation for T cell priming. pDCs constitutively express MHC class II molecules and lineage markers, such as CD45RA/B220$^+$, Ly6C/GR-1$^+$, and siglec-H [11–13]. Two cDCs subsets can be distinguished based on functional specialization. cDC1s are particularly efficient in CD8$^+$ T cell activation and cross-presentation. cDC2s are most efficient for CD4$^+$ T helper polarization, especially Th2 or Th17 [14]. In mice, cDC1s express high levels of CD8α or CD103 [15, 16] and cDC2s express CD11b and CD172a (also known as SIRPα) [17]. In humans, DCs can be subdivided into two main populations: CD141$^+$ DCs (also referred to as BDCA3$^+$) and CD1c$^+$ DCs (also known as BDCA1$^+$). Based on gene expression profiles and functions similarities, human CD141$^+$ DCs and CD1c$^+$ DCs resemble those of mouse cDC1s and cDC2s, respectively [18–21]. Also, monocytes can adopt a DC morphology and antigen-presenting functions in inflammatory sites, leading to their designation as monocytes-derived DCs (MoDCs) [22, 23]. In mice, MoDCs derived from Ly6C^{hi} monocytes can express CD11c and MHC class II, and, similarly to macrophages, F4/80 and CD64 [23, 24]. In humans, MoDCs derived from CD14$^+$ monocytes and can express CD1a [24]. Langerhans cells (LCs) present DC morphology and antigen-presenting functions in the skin [25, 26]. They constitutively express major histocompatibility complex (MHC) class II and high levels of the lectin Langerin [27]. The most current phenotypes described for each type of DC are summarized in Table 1.

Several reports show a central role for DCs in orchestrating immune responses in leishmaniasis [28–30]. In this review, we discuss the heterogeneity of the interaction between DCs and different species of *Leishmania* that causes CL.

2. Interaction of DC with Different *Leishmania* Species

Infection with *Leishmania* parasites leads to lifelong immunity against the same subspecies, after the infection is healed. Experimental models of CL infections are largely used to study the mechanism under this lifelong immunity. Most of these studies have been carried out by inoculation of *L. major*, a species present in the Old World. However, experimental studies with the New World *Leishmania* sp., such as *L. amazonensis* and *L. braziliensis*, are scarce. This reinforces the importance of studies about the immune response induced by specific species of *Leishmania*.

2.1. Interaction of DC Subtypes with Leishmania major. Current paradigms of the involvement of T helper subsets in infectious diseases are based, in large part, on the results of studies about resistance and susceptibility to *L. major* in inbred mice. In murine LCL, BALB/c mice respond to infection with production of Th2-type cytokines, in particular IL-4 and IL-10. These cytokines are associated with disease progression and susceptibility to *L. major*. In contrast, recovery from infection of resistant mice (e.g., C57BL/6) depends on the induction of a polarized Th1-type response, resulting in macrophage activation and killing of parasites.

Early studies demonstrated that epidermal LCs phagocyte *L. major in vivo* and migrate to draining lymph nodes (dLNs) for presentation to antigen-specific T cells [31]. However, later studies showed that DCs harboring parasites in dLNs are Langerin negative and express dermal DC markers [32]. Besides, mice deficient for MHC class II exclusively in LCs (but not in dermal DCs) control *L. major* infection, similar to wild type animals [33]. This finding suggests that LCs are dispensable for triggering T cell response during *Leishmania* infection. Moreover, a recent study showed that LCs might even play a pathogenic role during low dose infection via the induction and expansion of regulatory T cells [34]. Some studies showed that dermal DCs harboring parasites migrate out of the skin and transport antigens to the dLNs [32, 35]. Another study suggested that blood MoDCs might phagocyte parasites and transport them to the dLN, where they present parasite-derived antigen to T cells [29]. In this way, depending on the tissue and the subtype involved, DCs could have different biological response towards *Leishmania* interaction.

Table 1: Summary of current phenotypes described for different DC subsets.

DC type	Phenotype/markers	Function	Reference
Plasmacytoid DC (pDC)	MHC-II, CD45RA/B220, Ly6C/GR-1, Siglec-H	Type I-IFN synthesis	[11–13]
Conventional DC type 1 (cDC1)	CD8α, CD103 (mice); CD141/BDCA3 (humans)	Antigen cross-presentation, CD8α^+ T cell activation	[15, 16, 18, 19]
Conventional DC type 2 (cDC2)	CD11b, CD172/SIRPα (mice); CD1c/BDCA1 (humans)	CD4$^+$ T cell polarization	[17, 20, 21]
Monocyte-derived DC (MoDC)	CD11c, MHC-II, F4/80, CD64 (mice); CD1a (humans)	Antigen presentation at inflammatory sites	[23, 24]
Langerhans cell (LC)	MHC-II, Langerin	Antigen presentation in the skin	[25–27]

The production of IL-12 by APCs is critically important for the polarization of naïve T cells toward Th1 subset and subsequent IFN-γ production [30, 36]. Infection of DC with *L. major* results in functional IL-12p70 production [37]. Interestingly, DC subsets are differentially permissive to *Leishmania* parasites and this differential infectivity seems to be inversely correlated with the ability of infected cells to produce IL-12p70 [38, 39]. CD8α^+ DCs are less permissive to *L. major* amastigotes compared to CD8α^- DCs. However, CD8α^+ and CD103$^+$ DCs are the most powerful IL-12p70 producers in response to this infection [36, 38]. The mechanism(s) that control the induction of IL-12 from DCs and the functional differences between IL-12-producing DCs and nonproducers are still not known.

It has been speculated that different outcomes of *Leishmania* infection between resistant and susceptible mice may be related to differences in their DC functions, particularly in the differentiation of naïve TCD4$^+$ into effector cells [40, 41]. However, *L. major*-infected skin-derived DCs from BALB/c and C57BL/6 mice upregulated costimulatory molecules and produced comparable levels of proinflammatory cytokines [30]. In further contrast, LCs from BALB/c mice upregulate IL-4 receptor expression and downregulate IL-12p40 production in response to *L. major* infection [42]. These findings suggest that *L. major* is able to inhibit Th1 immune response through altering DCs functions, depending on the cell type involved. Baldwin et al. [43] found that *L. major*-infected BALB/c mice have an increased number of plasmacytoid DCs in their dLNs [43]. This was associated with increased pDC recruitment early after infection, compared to infected C57BL/6 mice.

Ashok and Acha-Orbea [44] proposed a model of infection based on DCs subtypes at the different time points after *L. major* infection. This model nicely explains many features and contradiction in the role of DCs subsets in cutaneous leishmaniasis: (1) dermal DCs and LCs play a role early in infection and (2) monocyte-derived dendritic cells and lymph node resident DCs are important to establish an efficient immune response at later time points [44]. However, this proposed model only focuses on DCs role in murine models based on *L. major* infection. It is not clear whether the differences observed in DCs from susceptible and resistant mice are relevant to the pathogenesis of the disease in humans. At present, there is still limited information on initial or late DC responses to other species of *Leishmania* and their contribution to prime protective or pathogenic T cell responses in cutaneous leishmaniasis.

2.2. Role of DCs Interaction with Other Leishmania Species. Even though cutaneous leishmaniasis is caused by almost 20 species of *Leishmania*, most studies about the role of DCs are focused on experimental models of 4 species: *L. major*, *L. mexicana*, *L. amazonensis, and L. braziliensis*.

The role of Langerhans cells (LCs) was examined in patients with different forms of cutaneous leishmaniasis (CL) caused by the New World *Leishmania* sp. (*L. braziliensis*, *L. mexicana*, *and L. amazonensis*) [45, 46]. The analysis of LCs density among different clinical forms of CL showed a reduced LC density in *L. braziliensis* infection with a positive DTH response (delayed type IV hypersensibility). In comparison to nonreactive DTH from severe forms caused by *L. amazonensis*, an increase of LC density was observed [46]. These results indicate a species-specific negative correlation between LC density and DTH reaction among clinical forms of CL. This could lead to a suppression of T cell immune response. However, in CL caused by *L. mexicana*, the LCs density is similar between mild and severe clinical forms [45]. These findings indicate that *L. amazonensis* may use LCs to prime regulatory T cells, inhibiting the T cell responses, in a similar way to *L. major* infection [34].

Moreover, corroborating this clinical observation, experimental evidence confirms that early stages of *L. amazonensis* infection in BALB/c mice may impair multiple immune functions, leading to an antigen-specific T cell immune suppression [47]. Similar results were observed in murine and human DCs infected *in vitro* by *L. amazonensis* [48, 49]. However, for *L. braziliensis* murine infection, a full DC maturation process and activation were observed [50]. Together, these studies point out the specificity of strategies from different *Leishmania* species to modulate T cell immune response through DCs. Besides, there is a lack of information about the importance of other DC types for the development of different clinical forms caused by one species.

The dynamics of DCs migration to lymph nodes and to nonlymphoid tissues is also an important issue for the disease outcome. DCs progenitors and monocytes terminally differentiate into DCs subsets, depending on the nonlymphoid tissue they migrate, such as the skin. When activated, skin DCs upregulate CCR7 and migrate again to draining

lymph node via afferent lymphatics in response to CCL19 and CCL21 [14, 51]. The migration of monocyte-derived DCs to the lymph nodes is driven by CCR2 and its ligands [52]. In VL, there is a lack of protective immune response, partially, due to an altered DC migration to the spleen and dLNs [53–56]. This is also observed in CL. During *L. major* infection, MoDCs are preferentially recruited to the infected skin and dLN. They are important to mediate a Th1 response and to control the infection [29]. Such enhanced recruitment of DCs to dLN leads to hypertrophy of the LN, which is associated with a protective response against *L. major* [57]. On the other hand, *L. mexicana* infection induces limited recruitment of MoDCs and decreased LN expansion, without affecting T cell proliferation [58, 59]. This diminished recruitment is independent of IL-10 and leads to disease progression, since treatment with neutralizing antibodies against IL-10 increases MoDCs migration and decreases parasite burden [59]. The modulation of DC recruitment to the infected skin and dLN could be used as a mechanism of immune evasion by different *Leishmania* sp. that causes CL.

3. Differences in Recognition of *Leishmania* Parasites by DCs

DCs express a wide variety of pattern recognition receptors (PRRs) that are important for initiating and directing subsequent adaptive immunity. The recognition of pathogen associated molecular patterns (PAMPs) can vary among species of *Leishmania*. de Veer et al. [60] found that MyD88 deficient ($MyD88^{-/-}$) C57BL/6 mice are more susceptible to *L. major* infection, suggesting a critical role of TLR signaling in initiating anti-*Leishmania* immunity [60]. They further demonstrated that LPG, the most abundant surface molecule of *Leishmania* and a TLR2 ligand, is responsible for the generation of protective immunity against leishmaniasis. Neutralization of TLR2 and TLR4 *in vivo* reduced the expression of costimulatory molecules on DCs infected with *L. major* [61]. However, the lack of TLR2 in mice infected with *L. braziliensis* resulted in an enhanced DC activation and increased IL-12 production. As such, *L. braziliensis*-infected DCs from $TLR2^{-/-}$ were more competent in priming naïve $CD4^+$ T cells *in vitro*. These findings correlated with an increased IFN-γ production *in vivo* and enhanced resistance to infection [62]. On the other hand, *L. braziliensis*-infected DCs from $MyD88^{-/-}$ exhibited less activation and decreased production of interleukin-12 [62].

Furthermore, it has been shown that TLR9 signaling is crucial to the release of IL-12 and type I IFN from DCs exposed *in vitro* to *L. major* and *L. braziliensis*. *In vivo* assays with *L. major* infection also confirmed the importance of TLR9 to IL-12 production from DCs [63, 64]. However, for *L. braziliensis* infection, *in vivo* experiments established that $TLR9^{-/-}$ mice could generate a Th1 response and activate DC, despite the diminished DC activation *in vitro* [65]. Together these data from TLR assays reinforce the importance to define *in vitro* and *in vivo* approaches to better characterize the modulation of DC induced by different *Leishmania* sp. on the immune response.

The recognition of pathogens could be optimized by the action of antibodies, a process called opsonization [66]. The uptake of *L. amazonensis* amastigotes by DC and LCs can be promoted by opsonization [67]. This process leads to IL-10 production from these DCs, as well as the consequent priming of IL-10-producing T $CD4^+$ and lesion progress in mice [67]. In contrast, the uptake of opsonized *L. major* by murine DCs leads to cell activation, IL-12 production, and protective immunity [68, 69]. In further contrast, *L. mexicana* and *L. braziliensis* are highly efficient in infecting DCs, even in the absence of antibodies [62, 70]. These findings point out that the profile of cytokine production from DC is differently induced in a species-specific way, despite the same pathway recognition of *Leishmania*.

4. Interaction of DC with Other Leukocytes

Dendritic cells are the most important APCs, making a link among innate and adaptive immunity. They can have direct and diverse functions on the immune response, leading to activation as well as tolerance and anergy. In the context of CL, the functions of DCs could be modulated by the interaction with other leukocytes, such as neutrophils and NK cells.

It has been shown that genomic DNA of *L. major* and *L. braziliensis* promastigotes activate cDCs and pDCs to produce IL-12 and IFN-α/β, respectively. After, they were cocultured with NK cells, leading to an increased IFN-γ release and NK cytotoxicity [63, 64]. Certain *Leishmania* species (*L. tropica*, *L. amazonensis*, and *L. mexicana*), in their amastigotes phase, are poor inducers of IL-12 by DC. This might account for the limited NK cell response during prolonged infections *in vivo* [71, 72]. Hernandez Sanabria et al. [73] demonstrated that infection of *L. amazonensis* amastigotes triggers minimal DC activation, but the interaction with activated NK cells could partially overcome the deficiencies in DC activation *in vitro* [73]. The injection of activated NK cells 24 hours after infection *in vivo* promoted IL-12 release and increased the expression of costimulatory molecules in infected DCs (CD40, CD83, and CD80) [73]. Regarding NK cells in this context, they showed increased expression of IFN-γ and CXCL10. Such interaction forms a positive loop, leading to the induction of a Th1 immune response to reduce parasite loads.

In a vaccination context against *L. major*, BALB/c depleted of NK cells and vaccinated with DCs pulsed with parasites lysates and, then, challenged with *L. major* showed a significant increase in footpad swelling and parasite load in the dLN [74]. In order to evaluate the mechanisms under this process, coculture of these cells was assessed. This resulted in upregulation of CD69 and IFN-γ on NK cells as well as CD86 and MHC-II on pulsed DCs. The interaction of DC and NK cells is a good example of a positive interaction that leads to cross activation and host immune protection, either, in the context of an infection or vaccination.

Neutrophils are also an important cell type which interact with DCs. The ingestion of *L. major* by neutrophils in parasite-inoculated mice increased cell apoptosis. This

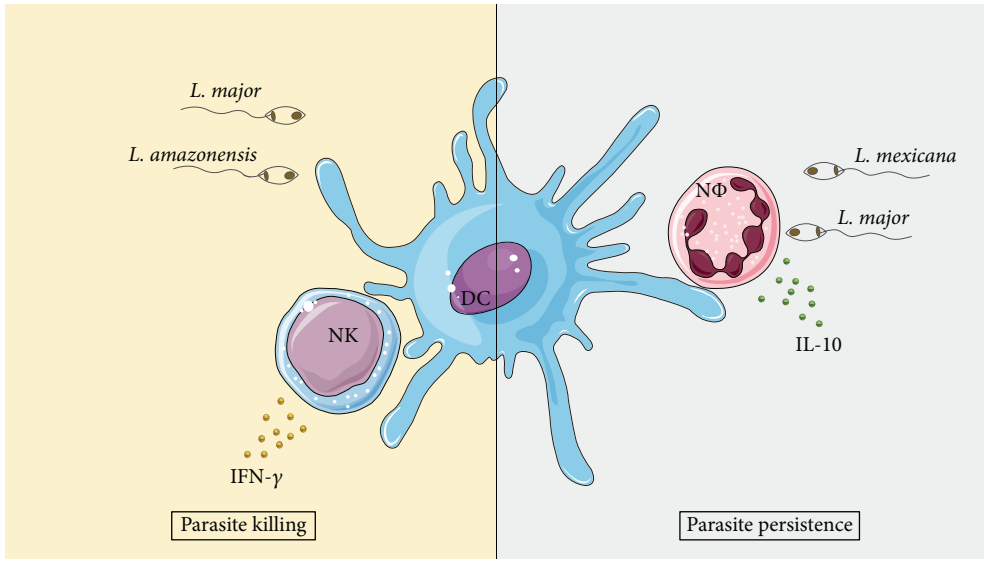


Figure 1: Interaction of DC with different leukocytes in the cutaneous leishmaniasis context. (a) shows the interaction among NK and infected DCs that leads to host immune protection and parasite killing through IFN-γ production during *L. amazonensis* [73] or *L. major* [74] infection. (b) shows the outcome induced by the increased production of IL-10 after the interaction between infected neutrophils (NΦ) with *L. major* [75] or *L. mexicana* [76] and DCs, leading to parasite persistence.

favored the capture of apoptotic neutrophils by DCs, preventing the activation of infected DCs in the skin [75]. In the case of *L. mexicana* infection, this effect was not observed, since the infection did not induce neutrophil apoptosis. Parasites sequestration by neutrophils impaired DC migration to the site of infection, through reduced CCL2, CCL3, and CCL5 release. Furthermore, the diminished DCs that migrate to the site of infection had a decreased motility and parasite uptake [76]. The interaction among DCs and neutrophils is a good example of negative regulation of the immune response, regardless of the *Leishmania* sp. Although the consequences of DCs and neutrophils interaction lead to immune suppression, the mechanisms of action could be diverse and *Leishmania* species-specific (summarized in Figure 1).

5. Systems Biology as a Tool to Develop Vaccines against CL Based on DCs

Recently, Matos et al. [77] showed the potential of targeting DC *in vivo* for induction of protective immune response against *L. major* in murine model of CL [77]. However, the role of DCs in CL is diverse and complex. Such role could explain some unique clinical manifestations, depending on the species of *Leishmania* that causes the disease. Because of that, the use of vaccines based on DCs is not yet a reality for CL. This is not only due to the complexity of the disease. Genomic and transcriptional profiles vary not only interspecifically in *Leishmania* parasites that causes CL [78–81], but also within parasites strains isolated from patients [82]. This variability leads to a difficult task to identify a universal antigen vaccine candidate to clinical trials.

Systems Biology could be a useful tool to overcome these difficulties. Systems Biology have a holistic approach to describe complex interactions between multiple components in a biological context [83]. Using high dimensional molecular approaches, Systems Biology identifies changes caused by perturbations, such as infection or vaccination, combined with computational analysis to model and predict responses [84]. The first studies of Systems Biology about the immune response predicted that certain signatures of $CD8^+$ T cells and B lymphocytes correlated with a protective immune response induced by a vaccine against Yellow Fever Virus [85, 86]. Since then, there is an increased interest about the research of immune responses based on Systems Biology approaches. These studies lead to the identification of interactions between pathogens and hosts and factors for parasite dissemination and disease progression, as well as to the selection of promising antigens as vaccine candidates [87–89]. For instance, hub genes with unknown functions were identified from *Plasmodium falciparum* parasites isolated from noncerebral clinical complications of malaria. The presence of these genes correlates parasite burden and survival with complicated clinical manifestations [90]. These findings revealed the crucial roles of these genes in parasite biology and their potential as candidates for intervention strategies.

Regarding leishmaniasis, Albergante et al. [91] have developed an *in silico* Petri net model that simulates hepatic granuloma development during the infection in experimental visceral context. This model identified an intergranuloma diversity of the antileishmanial activity and a dominant regulatory role of IL-10 produced by infected Kupffer cells at the core of the granuloma [91]. This approach raised new insights

into how effector mechanisms may be regulated within the granuloma and revealed a useful tool to interpret how interventions may operate. For cutaneous leishmaniasis, the analysis of DNA sequence of *L. braziliensis* and *L. guyanensis* isolated from patients with different treatment outcomes identified polymorphisms related to drug resistance [92]. This study showed that genes related to drug resistance could be used to discriminate the two species of the subgenus *L. Viannia* and also could predict treatment failure.

Those studies mentioned above demonstrate the use of System Biology as a useful tool to better understand an infection, to identify unknown pathogen cell signaling pathways, potential biomarkers of disease susceptibility, and immunological alterations that aggravates the pathology. However, the studies employing this approach are few, but they will be very promising for the development of new technologies on the leishmaniasis field.

A successful application of the System Biology approach was modeled to study the function and the role of pDC during cytopathic virus infection to identify multiscale interactions involved in the protection against the virus [93]. The results obtained from this analysis identified and predicted that (1) one infected pDC secretes sufficient type I IFN to protect up to 10^4 macrophages from cytopathic viral infection; (2) pDC population in the spleen protects against virus variants which inhibit IFN production; and (3) antiviral therapy should primarily limit viral replication within peripheral organs. Together, these results demonstrate the importance of System Biology application to direct and optimize the use of different technologies based on DCs.

In this way, the application of System Biology could be a useful tool to design and develop promising vaccines candidates based on DCs pulsed with *Leishmania* antigens. Studies about DC signaling network based on Systems Biology approach are already published and they stand for the feasibility of this technique [94, 95]. However, the development of vaccines based on DCs through Systems Biology approach needs to be well designed to avoid undesired effects, such as the exacerbation of the CL through the increase of inflammation [96].

6. Conclusion and Future Directions

Given the fact that the disease pathology of CL is highly variable depending on the species of *Leishmania*, it is very hard to generalize specific modulatory mechanisms to all strains and in all hosts. This is important because most of the studies about the role of DCs during *Leishmania* infection were usually conducted with a single species of the parasite, which precludes multi-species/strain comparison. Not all *Leishmania* species and its interaction with DCs were studied. For instance, infection caused by *L. guyanensis* paradoxically induces a specific immune response via TLR3 early after infection that impairs killing of parasites [97]. A more comprehensive study would be very helpful for a better understanding about the role of these cells and the mechanisms that regulate their antigen presentation functions and also pathogen factors that could influence the antigen presentation and subsequent activation of the adaptive immune system. Besides that, the development and use of computational immunology have been constantly increasing its value. Nowadays, different *in silico* approaches are available for identification of potential epitopes and antigens for vaccines, since experimental methods are difficult and time-consuming [98]. In addition, the DNA sequencing techniques became less expensive and, therefore, many parasite genome strains can be sequenced. Their predicted proteomes can be assessed considering their variability, an important feature of antigen candidates for vaccine development to one or all *Leishmania* species that cause CL. In this way, the use of DCs is promising for generation of potential alternatives therapies and vaccines protocols to improve the quality of life of patients infected by these protozoan parasites.

Conflict of Interests

The authors declare that they do not have a commercial association that might pose a conflict of interests.

Authors' Contribution

Cláudia Brodskyn and Natalia Tavares contributed equally to this review.

Acknowledgments

This review was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB), and Instituto de Investigação em Imunologia-Instituto Nacional de Ciência e Tecnologia (iii-INCT), Brazil. Daniel Feijó receives fellowship from CNPq. Cláudia Brodskyn is a senior investigator at CNPq. Rafael Tibúrcio and Mariana Ampuero receive fellowships from FAPESB. The authors thank Drs. Leonardo Farias, Bruno Andrade, and Pablo Oliveira for suggestions and comments. They also thank Elaine Santos and Andrezza Souza for technical support.

References

[1] WHO/Leishmaniasis, "Leishmaniasis: Magnitude of the problem," 2014, http://www.who.int/leishmaniasis/burden/magnitude/burden_magnitude/en/index.html.

[2] S. Saha, S. Mondal, A. Banerjee, J. Ghose, S. Bhowmick, and N. Ali, "Immune responses in kala-azar," *The Indian Journal of Medical Research*, vol. 123, no. 3, pp. 245–266, 2006.

[3] A. Caldas, C. Favali, D. Aquino et al., "Balance of IL-10 and interferon-γ plasma levels in human visceral leishmaniasis: implications in the pathogenesis," *BMC Infectious Diseases*, vol. 5, article 113, 2005.

[4] H. Goto and J. A. L. Lindoso, "Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis," *Expert Review of Anti-Infective Therapy*, vol. 8, no. 4, pp. 419–433, 2010.

[5] P. J. Guerin, P. Olliaro, S. Sundar et al., "Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda," *The Lancet Infectious Diseases*, vol. 2, no. 8, pp. 494–501, 2002.

[6] C. Reis e Sousa, "Activation of dendritic cells: translating innate into adaptive immunity," *Current Opinion in Immunology*, vol. 16, no. 1, pp. 21–25, 2004.

[7] H. Moll, "Dendritic cells and host resistance to infection," *Cellular Microbiology*, vol. 5, no. 8, pp. 493–500, 2003.

[8] M. Sundquist, A. Rydström, and M. J. Wick, "Immunity to *Salmonella* from a dendritic point of view," *Cellular Microbiology*, vol. 6, no. 1, pp. 1–11, 2004.

[9] T. B. H. Geijtenbeek, S. J. van Vliet, A. Engering, B. A. t Hart, and Y. van Kooyk, "Self- and nonself-recognition by C-type lectins on dendritic cells," *Annual Review of Immunology*, vol. 22, pp. 33–54, 2004.

[10] K. Takeda and S. Akira, "Microbial recognition by Toll-like receptors," *Journal of Dermatological Science*, vol. 34, no. 2, pp. 73–82, 2004.

[11] Y.-J. Liu, "IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors," *Annual Review of Immunology*, vol. 23, pp. 275–306, 2005.

[12] K. Shortman and Y.-J. Liu, "Mouse and human dendritic cell subtypes," *Nature Reviews Immunology*, vol. 2, no. 3, pp. 151–161, 2002.

[13] J. Zhang, A. Raper, N. Sugita et al., "Characterization of Siglec-H as a novel endocytic receptor expressed on murine plasmacytoid dendritic cell precursors," *Blood*, vol. 107, no. 9, pp. 3600–3608, 2006.

[14] M. Merad, P. Sathe, J. Helft, J. Miller, and A. Mortha, "The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting," *Annual Review of Immunology*, vol. 31, pp. 563–604, 2013.

[15] K. Crozat, R. Guiton, V. Contreras et al., "The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse CD8α^+ dendritic cells," *The Journal of Experimental Medicine*, vol. 207, no. 6, pp. 1283–1292, 2010.

[16] M. Guilliams, F. Ginhoux, C. Jakubzick et al., "Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny," *Nature Reviews Immunology*, vol. 14, no. 8, pp. 571–578, 2014.

[17] S. Gurka, E. Hartung, M. Becker, and R. A. Kroczek, "Mouse conventional dendritic cells can be universally classified based on the mutually exclusive expression of XCR1 and SIRPα," *Frontiers in Immunology*, vol. 6, article 35, 2015.

[18] M. Haniffa, A. Shin, V. Bigley et al., "Human tissues contain CD141^{hi} cross-presenting dendritic cells with functional homology to mouse CD103$^+$ nonlymphoid dendritic cells," *Immunity*, vol. 37, no. 1, pp. 60–73, 2012.

[19] S. L. Jongbloed, A. J. Kassianos, K. J. McDonald et al., "Human CD141$^+$ (BDCA-3)$^+$ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens," *The Journal of Experimental Medicine*, vol. 207, no. 6, pp. 1247–1260, 2010.

[20] S. H. Robbins, T. Walzer, D. Dembélé et al., "Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling," *Genome Biology*, vol. 9, no. 1, article R17, 2008.

[21] A. Schlitzer, N. McGovern, P. Teo et al., "IRF4 transcription factor-dependent CD11b$^+$ dendritic cells in human and mouse control mucosal IL-17 cytokine responses," *Immunity*, vol. 38, no. 5, pp. 970–983, 2013.

[22] C. Cheong, I. Matos, J.-H. Choi et al., "Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209$^+$ dendritic cells for immune T cell areas," *Cell*, vol. 143, no. 3, pp. 416–429, 2010.

[23] C. Langlet, S. Tamoutounour, S. Henri et al., "CD64 expression distinguishes monocyte-derived and conventional dendritic cells and reveals their distinct role during intramuscular immunization," *Journal of Immunology*, vol. 188, no. 4, pp. 1751–1760, 2012.

[24] S. Tamoutounour, M. Guilliams, F. M. Sanchis et al., "Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin," *Immunity*, vol. 39, no. 5, pp. 925–938, 2013.

[25] M. Greter, I. Lelios, P. Pelczar et al., "Stroma-derived interleukin-34 controls the development and maintenance of langerhans cells and the maintenance of microglia," *Immunity*, vol. 37, no. 6, pp. 1050–1060, 2012.

[26] J. C. Miller, B. D. Brown, T. Shay et al., "Deciphering the transcriptional network of the dendritic cell lineage," *Nature Immunology*, vol. 13, no. 9, pp. 888–899, 2012.

[27] M. Merad and M. G. Manz, "Dendritic cell homeostasis," *Blood*, vol. 113, no. 15, pp. 3418–3427, 2009.

[28] P. M. A. Gorak, C. R. Engwerda, and P. M. Kaye, "Dendritic cells, but not macrophages, produce IL-12 immediately following *Leishmania donovani* infection," *European Journal of Immunology*, vol. 28, no. 2, pp. 687–695, 1998.

[29] B. León, M. López-Bravo, and C. Ardavín, "Monocyte-derived dendritic cells formed at the infection site control the induction of protective T helper 1 responses against *Leishmania*," *Immunity*, vol. 26, no. 4, pp. 519–531, 2007.

[30] E. von Stebut, Y. Belkaid, T. Jakob, D. L. Sacks, and M. C. Udey, "Uptake of *Leishmania major* amastigotes results in activation and interleukin 12 release from murine skin-derived dendritic cells: implications for the initiation of anti-*Leishmania* immunity," *The Journal of Experimental Medicine*, vol. 188, no. 8, pp. 1547–1552, 1998.

[31] H. Moll, H. Fuchs, C. Blank, and M. Rollinghoff, "Langerhans cells transport *Leishmania* major from the infected skin to the draining lymph node for presentation to antigen-specific T cells," *European Journal of Immunology*, vol. 23, no. 7, pp. 1595–1601, 1993.

[32] U. Ritter, A. Meißner, C. Scheidig, and H. Körner, "CD8α- and Langerin-negative dendritic cells, but not Langerhans cells, act as principal antigen-presenting cells in leishmaniasis," *European Journal of Immunology*, vol. 34, no. 6, pp. 1542–1550, 2004.

[33] M. P. Lemos, F. Esquivel, P. Scott, and T. M. Laufer, "MHC class II expression restricted to CD8α^+ and CD11b$^+$ dendritic cells is sufficient for control of *Leishmania* major," *Journal of Experimental Medicine*, vol. 199, no. 5, pp. 725–730, 2004.

[34] K. Kautz-Neu, M. Noordegraaf, S. Dinges et al., "Langerhans cells are negative regulators of the anti-*Leishmania* response," *The Journal of Experimental Medicine*, vol. 208, no. 5, pp. 885–891, 2011.

[35] L. G. Ng, A. Hsu, M. A. Mandell et al., "Migratory dermal dendritic cells act as rapid sensors of protozoan parasites," *PLoS Pathogens*, vol. 4, no. 11, Article ID e1000222, 2008.

[36] M. Martínez-López, S. Iborra, R. Conde-Garrosa, and D. Sancho, "Batf3-dependent CD103$^+$ dendritic cells are major producers of IL-12 that drive local Th1 immunity against *Leishmania major* infection in mice," *European Journal of Immunology*, vol. 45, no. 1, pp. 119–129, 2015.

[37] M. A. Marovich, M. A. McDowell, E. K. Thomas, and T. B. Nutman, "IL-12p70 production by Leishmania major-harboring human dendritic cells is a CD40/CD40 ligand-dependent process," *Journal of Immunology*, vol. 164, no. 11, pp. 5858–5865, 2000.

[38] S. Henri, J. Curtis, H. Hochrein, D. Vremec, K. Shortman, and E. Handman, "Hierarchy of susceptibility of dendritic cell subsets to infection by *Leishmania* major: inverse relationship to interleukin-12 production," *Infection and Immunity*, vol. 70, no. 7, pp. 3874–3880, 2002.

[39] M. Akbari, K. Honma, D. Kimura et al., "IRF4 in dendritic cells inhibits IL-12 production and controls TH1 immune responses against leishmania major," *Journal of Immunology*, vol. 192, no. 5, pp. 2271–2279, 2014.

[40] K. Suzue, S. Kobayashi, T. Takeuchi, M. Suzuki, and S. Koyasu, "Critical role of dendritic cells in determining the Th1/Th2 balance upon L*eishmania major* infection," *International Immunology*, vol. 20, no. 3, pp. 337–343, 2008.

[41] M. J. Girard-Madoux, K. Kautz-Neu, B. Lorenz, J. L. Ober-Blöbaum, E. von Stebut, and B. E. Clausen, "IL-10 signaling in dendritic cells attenuates anti-*Leishmania major* immunity without affecting protective memory responses," *Journal of Investigative Dermatology*, vol. 135, no. 11, pp. 2890–2894, 2015.

[42] H. Moll, A. Scharner, and E. Kämpgen, "Increased interleukin 4 (IL-4) receptor expression and IL-4-induced decrease in IL-12 production by langerhans cells infected with *Leishmania* major," *Infection and Immunity*, vol. 70, no. 3, pp. 1627–1630, 2002.

[43] T. M. Baldwin, C. Elso, J. Curtis, L. Buckingham, and E. Handman, "The site of *Leishmania* major infection determines disease severity and immune responses," *Infection and Immunity*, vol. 71, no. 12, pp. 6830–6834, 2003.

[44] D. Ashok and H. Acha-Orbea, "Timing is everything: dendritic cell subsets in murine *Leishmania* infection," *Trends in Parasitology*, vol. 30, no. 10, pp. 499–507, 2014.

[45] U. Ritter, H. Moll, T. Laskay et al., "Differential expression of chemokines in patients with localized and diffuse cutaneous American leishmaniasis," *The Journal of Infectious Diseases*, vol. 173, no. 3, pp. 699–709, 1996.

[46] M. B. Xavier, F. T. Silveira, S. Demachki, M. M. R. Ferreira, and J. L. M. do Nascimento, "American tegumentary leishmaniasis: a quantitative analysis of Langerhans cells presents important differences between *L. (L.) amazonensis* and *Viannia subgenus*," *Acta Tropica*, vol. 95, no. 1, pp. 67–73, 2005.

[47] J. Ji, J. Sun, and L. Soong, "Impaired expression of inflammatory cytokines and chemokines at early stages of infection with Leishmania amazonensis," *Infection and Immunity*, vol. 71, no. 8, pp. 4278–4288, 2003.

[48] C. Favali, N. Tavares, J. Clarêncio, A. Barral, M. Barral-Netto, and C. Brodskyn, "*Leishmania* amazonensis infection impairs differentiation and function of human dendritic cells," *Journal of Leukocyte Biology*, vol. 82, no. 6, pp. 1401–1406, 2007.

[49] L. Soong, "Modulation of dendritic cell function by leishmania parasites," *Journal of Immunology*, vol. 180, no. 7, pp. 4355–4360, 2008.

[50] A. K. Carvalho, F. T. Silveira, L. F. D. Passero, C. M. C. Gomes, C. E. P. Corbett, and M. D. Laurenti, "*Leishmania (V.) braziliensis* and *L. (L.) amazonensis* promote differential expression of dendritic cells and cellular immune response in murine model," *Parasite Immunology*, vol. 34, no. 8-9, pp. 395–403, 2012.

[51] L. Ohl, M. Mohaupt, N. Czeloth et al., "CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions," *Immunity*, vol. 21, no. 2, pp. 279–288, 2004.

[52] F. Geissmann, S. Gordon, D. A. Hume, A. M. Mowat, and G. J. Randolph, "Unravelling mononuclear phagocyte heterogeneity," *Nature Reviews Immunology*, vol. 10, no. 6, pp. 453–460, 2010.

[53] M. K. Ibrahim, J. L. Barnes, E. Y. Osorio et al., "Deficiency of lymph node-resident dendritic cells (DCs) and dysregulation of DC chemoattractants in a malnourished mouse model of *Leishmania donovani* infection," *Infection and Immunity*, vol. 82, no. 8, pp. 3098–3112, 2014.

[54] M. Ato, A. Maroof, S. Zubairi, H. Nakano, T. Kakiuchi, and P. M. Kaye, "Loss of dendritic cell migration and impaired resistance to *Leishmania donovani* infection in mice deficient in CCL19 and CCL21," *The Journal of Immunology*, vol. 176, no. 9, pp. 5486–5493, 2006.

[55] M. Ato, S. Stäger, C. R. Engwerda, and P. M. Kaye, "Defective CCR7 expression on dendritic cells contributes to the development of visceral leishmaniasis," *Nature Immunology*, vol. 3, no. 12, pp. 1185–1191, 2002.

[56] A. C. Stanley, J. E. Dalton, S. H. Rossotti et al., "VCAM-1 and VLA-4 modulate dendritic cell IL-12p40 production in experimental visceral leishmaniasis," *PLoS Pathogens*, vol. 4, no. 9, Article ID e1000158, 2008.

[57] L. P. Carvalho, P. M. Petritus, A. L. Trochtenberg et al., "Lymph node hypertrophy following *Leishmania major* infection is dependent on TLR9," *The Journal of Immunology*, vol. 188, no. 3, pp. 1394–1401, 2012.

[58] A. C. Hsu and P. Scott, "Leishmania mexicana infection induces impaired lymph node expansion and Th1 cell differentiation despite normal T cell proliferation," *The Journal of Immunology*, vol. 179, no. 12, pp. 8200–8207, 2007.

[59] P. M. Petritus, D. Manzoni-de-Almeida, C. Gimblet, C. Gonzalez Lombana, and P. Scott, "*Leishmania mexicana* mexicana induces limited recruitment and activation of monocytes and monocyte-derived dendritic cells early during infection," *PLoS Neglected Tropical Diseases*, vol. 6, no. 10, Article ID e1858, 2012.

[60] M. J. de Veer, J. M. Curtis, T. M. Baldwin et al., "MyD88 is essential for clearance of *Leishmania major*: possible role for lipophosphoglycan and Toll-like receptor 2 signaling," *European Journal of Immunology*, vol. 33, no. 10, pp. 2822–2831, 2003.

[61] M. Komai-Koma, D. Li, E. Wang, D. Vaughan, and D. Xu, "Anti-Toll-like receptor 2 and 4 antibodies suppress inflammatory response in mice," *Immunology*, vol. 143, no. 3, pp. 354–362, 2014.

[62] D. A. Vargas-Inchaustegui, W. Tai, L. Xin, A. E. Hogg, D. B. Corry, and L. Soong, "Distinct roles for MyD88 and toll-like receptor 2 during *Leishmania braziliensis* infection in mice," *Infection and Immunity*, vol. 77, no. 7, pp. 2948–2956, 2009.

[63] J. Liese, U. Schleicher, and C. Bogdan, "TLR9 signaling is essential for the innate NK cell response in murine cutaneous leishmaniasis," *European Journal of Immunology*, vol. 37, no. 12, pp. 3424–3434, 2007.

[64] U. Schleicher, J. Liese, I. Knippertz et al., "NK cell activation in visceral leishmaniasis requires TLR9, myeloid DCs, and IL-12, but is independent of plasmacytoid DCs," *The Journal of Experimental Medicine*, vol. 204, no. 4, pp. 893–906, 2007.

[65] T. Weinkopff, A. Mariotto, G. Simon et al., "Role of toll-like receptor 9 signaling in experimental *Leishmania braziliensis* infection," *Infection and Immunity*, vol. 81, no. 5, pp. 1575–1584, 2013.

[66] L. T. Vogelpoel, D. L. Baeten, E. C. de Jong, and J. den Dunnen, "Control of cytokine production by human fc gamma receptors: implications for pathogen defense and autoimmunity," *Frontiers in Immunology*, vol. 6, article 79, 2015.

[67] N. Wanasen, L. Xin, and L. Soong, "Pathogenic role of B cells and antibodies in murine *Leishmania amazonensis* infection,"

International Journal for Parasitology, vol. 38, no. 3-4, pp. 417–429, 2008.

[68] A. P. Nigg, S. Zahn, D. Rückerl et al., "Dendritic cell-derived IL-12p40 homodimer contributes to susceptibility in cutaneous leishmaniasis in BALB/c mice," *The Journal of Immunology*, vol. 178, no. 11, pp. 7251–7258, 2007.

[69] F. Woelbing, S. L. Kostka, K. Moelle et al., "Uptake of *Leishmania major* by dendritic cells is mediated by Fcγ receptors and facilitates acquisition of protective immunity," *The Journal of Experimental Medicine*, vol. 203, no. 1, pp. 177–188, 2006.

[70] E. Prina, S. Z. Abdi, M. Lebastard, E. Perret, N. Winter, and J.-C. Antoine, "Dendritic cells as host cells for the promastigote and amastigote stages of *Leishmania amazonensis*: the role of opsonins in parasite uptake and dendritic cell maturation," *Journal of Cell Science*, vol. 117, no. 2, pp. 315–325, 2004.

[71] C. L. Bennett, A. Misslitz, L. Colledge, T. Aebischer, and C. C. Blackburn, "Silent infection of bone marrow-derived dendritic cells by *Leishmania mexicana* amastigotes," *European Journal of Immunology*, vol. 31, no. 3, pp. 876–883, 2001.

[72] M. A. McDowell, M. Marovich, R. Lira, M. Braun, and D. Sacks, "Leishmania priming of human dendritic cells for CD40 ligand-induced interleukin-12p70 secretion is strain and species dependent," *Infection and Immunity*, vol. 70, no. 8, pp. 3994–4001, 2002.

[73] M. X. Hernandez Sanabria, D. A. Vargas-Inchaustegui, L. Xin, and L. Soong, "Role of natural killer cells in modulating dendritic cell responses to *Leishmania amazonensis* infection," *Infection and Immunity*, vol. 76, no. 11, pp. 5100–5109, 2008.

[74] K. A. Remer, B. Roeger, C. Hambrecht, and H. Moll, "Natural killer cells support the induction of protective immunity during dendritic cell-mediated vaccination against *Leishmania major*," *Immunology*, vol. 131, no. 4, pp. 570–582, 2010.

[75] F. L. Ribeiro-Gomes, N. C. Peters, A. Debrabant, and D. L. Sacks, "Efficient capture of infected neutrophils by dendritic cells in the skin inhibits the early anti-leishmania response," *PLoS Pathogens*, vol. 8, no. 2, Article ID e1002536, 2012.

[76] B. P. Hurrell, S. Schuster, E. Grün et al., "Rapid sequestration of *Leishmania mexicana* by neutrophils contributes to the development of chronic lesion," *PLoS Pathogens*, vol. 11, no. 5, Article ID e1004929, 2015.

[77] I. Matos, O. Mizenina, A. Lubkin, R. M. Steinman, and J. Idoyaga, "Targeting antigens to dendritic cells in vivo induces protective immunity," *PLoS ONE*, vol. 8, no. 6, Article ID e67453, 2013.

[78] L. A. Dillon, K. Okrah, V. K. Hughitt et al., "Transcriptomic profiling of gene expression and RNA processing during *Leishmania major* differentiation," *Nucleic Acids Research*, vol. 43, no. 14, pp. 6799–6813, 2015.

[79] M. Fiebig, S. Kelly, E. Gluenz, and P. J. Myler, "Comparative life cycle transcriptomics revises *Leishmania mexicana* genome annotation and links a chromosome duplication with parasitism of vertebrates," *PLoS Pathogens*, vol. 11, no. 10, Article ID e1005186, 2015.

[80] C. S. Peacock, K. Seeger, D. Harris et al., "Comparative genomic analysis of three *Leishmania* species that cause diverse human disease," *Nature Genetics*, vol. 39, no. 7, pp. 839–847, 2007.

[81] D. A. Tschoeke, G. L. Nunes, R. Jardim et al., "The comparative genomics and phylogenomics of *Leishmania amazonensis* parasite," *Evolutionary Bioinformatics*, vol. 10, pp. 131–153, 2014.

[82] E. V. Alves-Ferreira, J. S. Toledo, A. H. De Oliveira et al., "Differential gene expression and infection profiles of cutaneous and mucosal *Leishmania braziliensis* isolates from the same patient," *PLoS Neglected Tropical Diseases*, vol. 9, no. 9, Article ID e0004018, 2015.

[83] L. H. Hartwell, J. J. Hopfield, S. Leibler, and A. W. Murray, "From molecular to modular cell biology," *Nature*, vol. 402, no. 6761, supplement, pp. C47–C52, 1999.

[84] H. Kitano, "Systems biology: a brief overview," *Science*, vol. 295, no. 5560, pp. 1662–1664, 2002.

[85] D. Gaucher, R. Therrien, N. Kettaf et al., "Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses," *The Journal of Experimental Medicine*, vol. 205, no. 13, pp. 3119–3131, 2008.

[86] T. D. Querec, R. S. Akondy, E. K. Lee et al., "Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans," *Nature Immunology*, vol. 10, no. 1, pp. 116–125, 2009.

[87] C. J. Blohmke, D. O'Connor, and A. J. Pollard, "The use of systems biology and immunological big data to guide vaccine development," *Genome Medicine*, vol. 7, no. 1, article 114, 2015.

[88] J. B. Gutierrez, M. R. Galinski, S. Cantrell, and E. O. Voit, "From within host dynamics to the epidemiology of infectious disease: scientific overview and challenges," *Mathematical Biosciences B*, vol. 270, pp. 143–155, 2015.

[89] D. E. Zak and A. Aderem, "Systems integration of innate and adaptive immunity," *Vaccine*, vol. 33, no. 40, pp. 5241–5248, 2015.

[90] A. K. Subudhi, P. A. Boopathi, I. Pandey et al., "Disease specific modules and hub genes for intervention strategies: a co-expression network based approach for *Plasmodium falciparum* clinical isolates," *Infection, Genetics and Evolution*, vol. 35, pp. 96–108, 2015.

[91] L. Albergante, J. Timmis, L. Beattie, and P. M. Kaye, "A Petri net model of granulomatous inflammation: implications for IL-10 mediated control of *Leishmania donovani* infection," *PLoS Computational Biology*, vol. 9, no. 11, Article ID e1003334, 2013.

[92] D. C. Torres, M. Ribeiro-Alves, G. A. S. Romero, A. M. R. Dávila, and E. Cupolillo, "Assessment of drug resistance related genes as candidate markers for treatment outcome prediction of cutaneous leishmaniasis in Brazil," *Acta Tropica*, vol. 126, no. 2, pp. 132–141, 2013.

[93] G. Bocharov, R. Züst, L. Cervantes-Barragan et al., "A systems immunology approach to plasmacytoid dendritic cell function in cytopathic virus infections," *PLoS Pathogens*, vol. 6, no. 7, Article ID e1001017, pp. 1–15, 2010.

[94] D. Cavalieri, D. Rivero, L. Beltrame et al., "DC-ATLAS: a systems biology resource to dissect receptor specific signal transduction in dendritic cells," *Immunome Research*, vol. 6, no. 1, article 10, 2010.

[95] S. Patil, H. Pincas, J. Seto, G. Nudelman, I. Nudelman, and S. C. Sealfon, "Signaling network of dendritic cells in response to pathogens: a community-input supported knowledgebase," *BMC Systems Biology*, vol. 4, article 137, 2010.

[96] P. Tsagozis, E. Karagouni, and E. Dotsika, "Dendritic cells pulsed with peptides of gp63 induce differential protection against experimental cutaneous leishmaniasis," *International Journal of Immunopathology and Pharmacology*, vol. 17, no. 3, pp. 343–352, 2004.

[97] A. Ives, C. Ronet, F. Prevel et al., "Leishmania RNA virus controls the severity of mucocutaneous leishmaniasis," *Science*, vol. 331, no. 6018, pp. 775–778, 2011.

[98] D. R. Flower, I. K. Macdonald, K. Ramakrishnan, M. N. Davies, and I. A. Doytchinova, "Computer aided selection of candidate vaccine antigens," *Immunome Research*, vol. 6, supplement 2, article S1, 2010.

Hindawi Publishing Corporation
Journal of Immunology Research
Volume 2015, Article ID 450695, 8 pages
http://dx.doi.org/10.1155/2015/450695

Review Article

MGL Receptor and Immunity: When the Ligand Can Make the Difference

Ilaria Grazia Zizzari, Chiara Napoletano, Federico Battisti, Hassan Rahimi, Salvatore Caponnetto, Luca Pierelli, Marianna Nuti, and Aurelia Rughetti

Department of Experimental Medicine, "Sapienza" University, Viale Regina Elena 324, 00161 Rome, Italy

Correspondence should be addressed to Marianna Nuti; marianna.nuti@uniroma1.it

Received 23 October 2015; Accepted 10 December 2015

Academic Editor: Christine Trumpfheller

C-type lectin receptors (CLRs) on antigen-presenting cells (APCs) facilitate uptake of carbohydrate antigens for antigen presentation, modulating the immune response in infection, homeostasis, autoimmunity, allergy, and cancer. In this review, we focus on the role of the macrophage galactose type C-type lectin (MGL) in the immune response against self-antigens, pathogens, and tumor associated antigens (TAA). MGL is a CLR exclusively expressed by dendritic cells (DCs) and activated macrophages (MØs), able to recognize terminal GalNAc residues, including the sialylated and nonsialylated Tn antigens. We discuss the effects on DC function induced throughout the engagement of MGL, highlighting the importance of the antigen structure in the modulation of immune response. Indeed modifying Tn-density, the length, and steric structure of the Tn-antigens can result in generating immunogens that can efficiently bind to MGL, strongly activate DCs, mimic the effects of a danger signal, and achieve an efficient presentation in HLA classes I and II compartments.

1. Introduction

Dendritic cells (DCs), as professional antigen-presenting cells (APCs), sense the microenvironment through different types of receptors to scan local environmental changes and eliminate incoming pathogens [1]. They play an essential role in the uptake of self- or pathogen-associated antigens, thus, steering and directing the immune response. After activation, DCs migrate to the draining lymph nodes, where they initiate specific immunity. DCs, similarly to macrophages (MØs) and B cells, are equipped with a set of receptors that recognize, capture, and internalize foreign antigens to facilitate an efficient processing and presentation through MHC II and I molecules. While B cells are specialized to recognize an extensive variety of epitopes due to the presence of somatically variable surface immunoglobulin receptors, DCs and MØs rely on a set of germline-encoded membrane receptors for the discrimination and recognition of antigenic determinants. Besides complement and Fc receptors, DCs express a large array of pattern-recognition receptors (PRRs), which have evolved to activate and modulate immune functions upon encountering ligands from "nonself" (pathogen-associated molecular patterns (PAMPs)), "damaged self" (damage-associated molecular patterns (DAMPs)), or "altered self" as in cancer (tumor-associated molecular patterns (TAMPs)) [2, 3]. The PRRs are a heterogeneous group of receptor subfamilies, among which the best characterized are the toll-like receptors (TLRs) and the C-type lectin receptors (CLRs). The TLRs respond to a wide variety of pathogen-derived molecular structures with a response characterized by the activation of proinflammatory signaling pathways [4]. However, TLRs are not able to internalize antigens. This function is instead largely covered by CLRs. CLRs were initially thought to function only as scavenger receptors able to bind various pathogens upon recognition of particular carbohydrate profiles through at least one carbohydrate recognition domain (CRD). CLRs recognize and internalize specific carbohydrate antigens in Ca^{2+}-dependent manner [5] thus influencing the outcome of the immune response [6]. In fact, the importance of C-type lectins is highlighted by the fact that these receptors are able to trigger numerous cellular and immunological responses critical for the control and

Most common human CLRs

CLR	CLR type	Carbohydrate specificity
MR	I	High mannose, fucose, and sulphated sugars (sLex)
DEC-205	I	ND
MGL	II	Terminal GalNAc (Tn antigen, LDN) and sialyl-Tn (STn)
Dectin-1	II	β-1,3 glucans
DC-SIGN	II	High mannose and fucose (Le^X, Le^Y, Le^A, Le^B)
Langerin	II	High mannose, fucose (Le^Y, Le^B), and GlcNAc

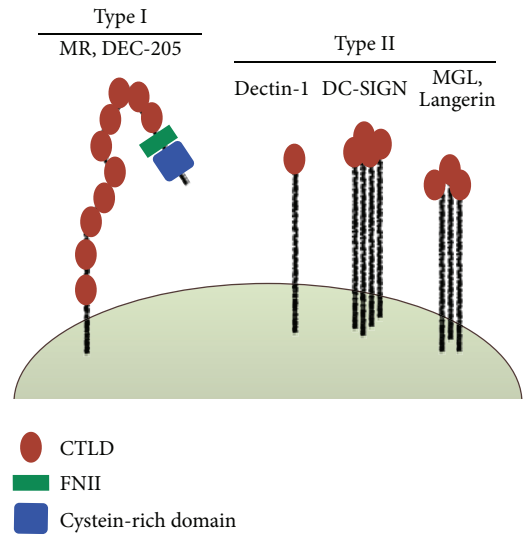


Figure 1: Schematic representation of type I and type II C-type lectins and lectin-like receptors. Type I CLRs (MR, DEC-205) are composed of a N-terminal cysteine-rich domain, a single fibronectin type II (FNII) domain, and 8–10 CTLDs all expressing CRDs. Type II CLRs (Langerin, DC-SIGN, MGL) or lectin-like receptors (Dectin-1) are composed of a single CTLD, an extracellular stalk region, a transmembrane region, and a N-terminal cytoplasmic tail with or without a signaling motif or proline-rich region. Langerin, DC-SIGN, MGL, and Dectin-1 express a CRD on their CTLD. CTLD, C-type lectin like domain; CLR, C-type lectin receptor; CRD, carbohydrate recognition domain; MR, mannose receptor; DC-SIGN, DC-specific ICAM3-grabbing nonintegrin; MGL, macrophage galactose type C-type lectin.

regulation of infection, homeostasis, autoimmunity, allergy, and cancer [7–9]. Several studies have demonstrated that some C-type lectins may function as adhesion, signaling, or antigen-uptake receptors [10–12]. These results are consistent with the fact that CLRs are present on MØs and DCs, which play a role in the initial step of capturing the antigens carrying carbohydrates [13]. Pathogens recognition by CLRs leads to its internalization, degradation, and subsequent antigen presentation. Besides antigen recognition and internalization, CLRs are also able to induce intracellular signaling and recruit other molecules such as TLRs that can modulate the signaling cascade [14]. In particular, CLR triggering by different pathogens can induce diverse immune responses [8]. For this reason and for their potential implication in the therapy of immune diseases and cancer, this receptor family has received great attention in recent years.

The most important molecules from the CLR family include macrophage galactose type C-type lectin (MGL), dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN), mannose receptor (MR), DEC205, Dectin-1, and Langerin. These receptors are able to trigger distinct signaling pathways that modulate APC functions through the expression of specific molecules and cytokines, determining the polarization of T cells [8].

CLRs such as DC-SIGN, MGL, and Langerin are well characterized for their specificity for high-mannose, fucose-containing glycans ($Lewis^{A,B,X,Y}$), GalNAc (N-Acetylgalactosamine) and high mannose, fucose ($Lewis^Y$, $Lewis^B$), and GlcNAc (N-Acetylglucosamine), respectively (Figure 1) [15, 16]. These glycan structures can be expressed by both mammalian cells and pathogens, reflecting CLR dual function in host-pathogen recognition and immune cell responses.

The APCs are the first line of defence responsible for clearing pathogens and they subsequently initiate adaptive immune responses. The DC-expressed C-type lectins mannose receptor, DC-SIGN, MGL, Dectin-1, and Langerin on Langherans cells are involved in glycan-mediated pathogen recognition and internalization of antigen for loading on MHC class I and class II molecules, thereby facilitating effective antigen-specific $CD4^+$ and $CD8^+$ T cell responses [17]. Thus, most C-type lectins are antigen-uptake receptors that facilitate MHC-restricted antigen presentation to T cells. All these lectins are considered powerful signaling molecules that positively or negatively instruct DC differentiation and subsequent T cell responses.

In this review, we focus on the role of human MGL as prototype receptor highly specialized in the glycan recognition in immune system, on its high plasticity and capacity to modulate the immune response conditioned by the type of the ligand.

2. Human MGL in Immune Response

MGL is a CLR exclusively expressed *in vivo* by human DCs of skin and lymph nodes and *in vitro* by macrophages and monocytes derived DCs [18, 19]. Within the CLR family, MGL is the only CLR within the human immune system that exclusively recognizes terminal α- or β N-acetylgalactosamine (GalNAc or Tn) residues [20, 21]. In particular, its carbohydrate recognition domains contain a QPD sequence that is responsible for recognition of GalNAc/Tn residues of *N*- and *O*-glycans carried by glycoproteins and/or glycosphingolipids of helminths, bacteria, filovirus, and tumor-associated antigens [20].

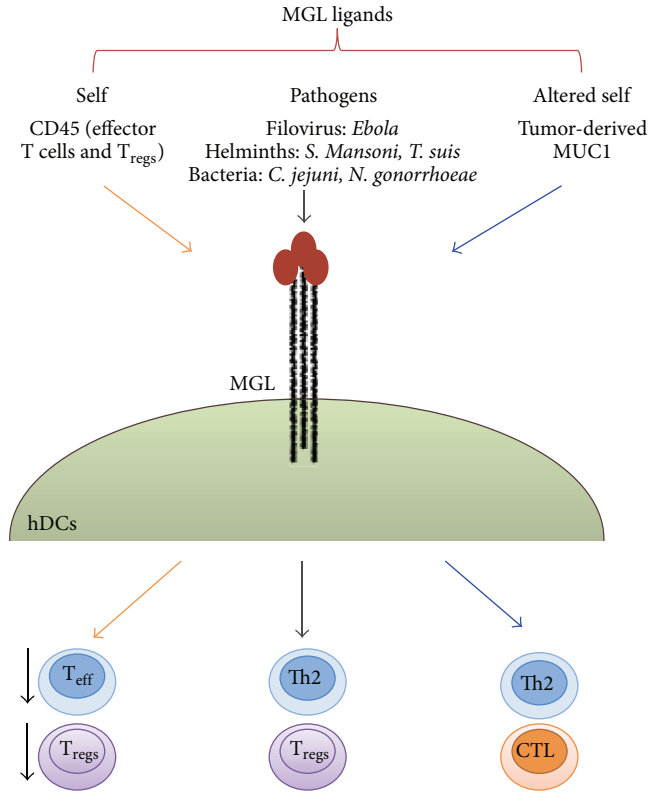


Figure 2: Representation of ligands binding human MGL expressed by DCs and impact of MGL triggering on immune response.

In mice, there are two homologs of human MGL, MGL1, and MGL2 [22], expressed by dermal DCs and alternatively activated macrophages [18, 23]. MGL1 is highly specific for Lewis X and Lewis A structures, while MGL2, similar to the human MGL, recognizes *N*-GalNAc and galactose and *O*-linked Tn-antigen, TF-antigen, and core 2 structures [24].

Human MGL is equipped with a partial dileucine zipper, with YENF internalization motifs [25], and is involved in (a) the recognition of a large plethora of pathogens by DCs [26, 27], (b) in the maintenance of homeostasis [28], and (c) in the interaction with TAA derived from aberrant glycosylation processes [16, 29]. More recently, our group has also demonstrated the role of MGL in immunosuppression given by its capacity to modulate regulatory T cell function [30] (Figure 2). Thus MGL acts as promiscuous receptor that can bind more than one ligand modulating various types of immune response. The pathogens that bind human MGL include *Ebola* virus through the interaction with the viral envelope protein GP2. It was demonstrated that this lectin promotes filovirus entry suggesting a role for MGL in viral replication *in vivo* [31]. Also the helminths *Schistosoma mansoni* and *Trichuris suis* interact with MGL [32, 33]. Schistosoma soluble egg antigens (SEA) consistently are inducers of Th2 responses in different experimental settings either *in vitro* or *in vivo* and both in humans or in animal models. In particular, studies performed on DCs have been instrumental in understanding the polarization of immune responses towards Th2 by SEA [32, 34–38]. While DCs fail to show classic signs of maturation when stimulated with SEA [32, 34], *in vitro* experiments show that SEA-primed monocyte-derived DCs (both human and murine) are very potent in polarizing naive Th cells towards a Th2 type [34, 35, 38].

DCs show remarkable phenotypic changes when recognizing soluble products (SPs) of *Trichuris suis*, a pig whipworm that is experimentally used in therapies to ameliorate inflammation in patients with Crohn's disease and multiple sclerosis [39]. *Trichuris suis* glycans play an important role in the capacity to suppress proinflammatory cytokine and chemokine production of DCs interacting with DCs via CLRs, such as MGL. In particular, *T. suis* SPs suppress the production of the proinflammatory cytokines, IL-12, TNF-α, and IL-6, and many proinflammatory chemokines. These properties, in combination with upregulation of OX40L and CXCL16 expression, are regarded as positive signals for Th2 polarization [40, 41].

Human MGL, through GalNAc-terminated lipopolysaccharide (LPS) and glycoproteins, interacts also with bacteria such as *Campylobacter jejuni* and *Neisseria gonorrhoeae* [26, 27]. *N. gonorrhoeae* phenotype C, carrying a terminal N-acetylgalactosamine, primarily interacted with MGL and skewed immunity towards the Th2 lineage through IL-4 production, whereas *N. gonorrhoeae* variant A with a terminal N-acetylglucosamine on its lipooligosaccharide (LOS) was recognized by DC-SIGN and induced significantly more IL-10 production.

In humans, monocyte-derived DCs express moderate MGL levels, which become negative after DC maturation. In addition variation of its expression related to seasonal changes is observed [42]. Moreover, MGL is upregulated on tolerogenic DCs generated in the presence of glucocorticoids and during chronic inflammatory conditions such as rheumatoid arthritis, implicating MGL in immune regulation [43]. In fact MGL has been shown to interact directly with a subset of $CD4^+$ and/or $CD8^+$ effector T cells. The MGL ligand on these T cells was identified as CD45, which exposes terminal GalNAc (Tn) structures. This interaction negatively regulates T cell receptor-mediated signaling by decreasing the phosphatase activity of CD45 and inhibiting lymphocyte protein tyrosine kinase (Lck) activation and Ca^{2+} mobilization [9] that results in enhanced apoptosis of T cells and reduced secretion of proinflammatory cytokines [9, 43]. We have more recently demonstrated that also $CD45RA^+$ Treg subpopulation is affected by MGL engagement. CD45RA-MGL cross-linking induces a decrease of Treg immunosuppressive activity by affecting CD45RA and TCR signaling and an increase of Foxp3 methylation accompanied by a reduced production of suppressive cytokines [30]. Recent evidence indicates that human MGL expressed on DCs is also able to generate antigen specific IL-10 producing $CD4^+$ T cells when stimulated with foreign and self-antigens fused to an anti-MGL antibody [44].

These results demonstrate an important function for MGL in the regulation of T cell homeostasis and in the silencing of potentially harmful T cell activation.

3. MGL in Cancer Immunity

The capacity of CLRs to bind, process, and cross-present antigens has received much attention in the field of cancer immunity. Several of tumor-related glycoforms of self-antigens are in fact specific ligands for CLRs expressed on DCs, such as MGL. These evidences open up a new area of research to investigate whether these tumor specific glycoforms affect CLR signaling and DC differentiation, thereby modulating innate and adaptive antitumor response. MGL is able to recognize the mucin MUC1, an *O*-linked glycosylated transmembrane protein normally expressed on the apical surface of epithelial cells, but aberrantly expressed in a broad spectrum of carcinomas. Upon malignant transformation, MUC1 loses polarity and becomes overexpressed and aberrantly glycosylated, revealing an immunogenic region of tandem repeats of 20 residues. The novel MUC1 glycoforms that arise carry shortened glycan moieties: Tn (GalNAc), T (Galβ1, 3GalNAc), ST (NeuAcα2, 3Galβ1, and 3GalNAc), and STn (NeuAcα2, 6GalNAc) [20, 45]. Because MGL recognizes GalNAc-containing epitopes frequently expressed on the surface of cancer cells and is involved in the regulation of the adaptive and innate immune response, it was chosen as a probe for glycoprofiling in breast cancer. Results indicate that high MGL-binding molecules in breast were associated with the expression of HER2/neu [46, 47]. In particular, it was demonstrate that detection of Tn ligands in mammary tissue is feasible employing the MGL recombinant protein and that this experimental approach permits recognizing posttranslational modifications (PTMs) such as phosphorylation and glycosylation. The identification of tumor-associated glycans, which potentially interact with the CRDs, could become a tool to identify those patients who will profit from MGL based specific therapeutic approaches. Tn antigen has previously been associated with worse survival [48, 49] and recent research indicates that tumor-specific Tn expression not only promotes tumor cell invasiveness [50] but also alters the immunogenicity of tumor antigens [51]. MGL is able to distinguish healthy tissue from tumor through its specific recognition of Tn antigen. *In vitro* studies, using CRC cell lines, showed an association between MGL ligand expression and the presence of BRAFV600E, suggesting a model in which activating BRAF mutations, and possibly other oncogenic alterations that activate the MAPK pathway, lead to an altered tumor cell glycosylation profile and enhanced expression of MGL ligands [48]. These aberrant glycans on tumor cells may have the ability to suppress antitumor immune responses through activation of the MGL receptor on DC. In fact, the prognostic value of MGL-binding to tumor cells is predominantly evident in stage III of colon cancer patients and not in stage II patients, that is, when tumor cells are no longer confined to the intestine but have spread into the local lymph nodes.

Recently MGL has been shown to bind also STn [21, 52, 53]. Although the binding of glycoproteins carrying Tn has been investigated [16, 54], the actual role of STn-carrying proteins binding to MGL is still to be fully investigated. Highly purified recombinant human MUC1 glycoproteins and MUC1 glycopeptides carrying either Tn or STn glycans bind MGL expressed by immature monocyte-derived DCs and by K562 transfected with MGL [52]. The interaction with the two glycoforms displays a similar affinity as demonstrated by atomic force microscopy (AFM). This is important because, although the vast majority of breast cancers stain for Tn [50], the 20–25% of breast cancer express the STn glycoforms on plasma membrane [54].

The most relevant result in tumor is the interaction between MGL, expressed by DCs, and tumor through the Tn glycans expressed by MUC1 tumor associated antigen [16, 29, 55]. The signaling activated by MGL has been recently well characterized in DCs. The MGL engagement with an anti-MGL antibody or MUC1-Tn glycopeptide (60 amino acids) triggers the extracellular signal-regulated kinases 1 and 2 (ERK1,2) and nuclear factor-κB (NF-κB) pathways and induces phenotypic and functional DC maturation to license DCs to initiate a strong $CD8^+$ T cell immune response [55]. Moreover, similar to other CLRs, MGL signaling synergies with TLR2-induced pathways in DCs, leading to elevated IL-10 mRNA levels and enhanced TNF-α mRNA stability. In addition, MGL signaling promoted phosphorylation of the MAPK ERK1,2 and the transcription factor CREB. At the same time, NF-κB seems to be crucial for the IL-10 response and dispensable for TNF-α production. Together, these results demonstrate that MGL activation modulates DC maturation and this ability highlights the possibility to use this receptor as a target for anticancer vaccination strategies.

4. The Nature of the Ligand Can Modulate Signals through MGL

The coupling of CLRs to different signal transduction modules is influenced not only by the receptor but also by the nature, density, size, and architecture of the ligand, which can affect the rate of receptor internalization and trafficking to different intracellular compartments. Understanding how the variety of ligands can trigger differential CLR signaling and function represents a fascinating biological challenge.

MGL was first described as a C-Type lectin with high endocytic activity regulated by the "YENF" consensus sequence contained in the cytoplasmic tail. MGL mediated endocytosis of soluble Tn-carrying antigens resulted in processing in HLAII compartment and activation of antigen specific $CD4^+$ T cells [25, 56]. However these reports did not investigate the processing in HLAI compartment and the possible induction of $CD8^+$ T cells responses. The effects on DCs induced by MGL engagement differ on the basis of ligands. Employing the Tn-MUC1 glycoform as cancer model glycoantigen, we showed that the structure of the MGL ligand dictates the upcoming intracellular processing. In fact, upon MGL engagement, the large soluble recombinant Tn-MUC1 glycoprotein (Tn-$MUC1_{16TR}$) corresponding to the one shed *in vivo* by epithelial cancers remained trapped in the endolysosomal/HLAII compartment, while the MUC1 peptide (Tn-$MUC1_{3TR}$), 60 amino acids long, carrying 9 Tn moles colocalized both in HLAII and HLAI compartments [16, 57] (Figure 3(a)).

It is interesting to note that this distinct intracellular routing of the two MUC1 based ligands matches with a distinct signaling and phenotypic profile of DCs induced by MGL engagement.

In fact, cross-processing in HLAI compartment of the 9Tn-$MUC1_{3TR}$ is accompanied by triggering of ERK1,2 phosphorylation, activation of NF-κB signal, and upregulation of maturative markers. These effects are similar, although weaker, to the ones induced by a strong ligand as a specific anti-MGL MoAb [55], suggesting that MGL engagement could be used as adjuvant in DC-based vaccination (Figure 3(b)). On the other hand, preliminary and unpublished results from our laboratory indicate that the MGL mediated endocytosis of the large soluble Tn-MUC1 molecule, retained in HLAII compartment, does not modify the balance of ERK1,2/NFkB and DC phenotype. How the different structure of the ligand and therefore its avidity and affinity can modulate intracellular pathway activated by MGL cytoplasmic tail is yet to be defined. For other C-type lectins it has been proposed that ligand affinity and avidity as well as the particulate form of the ligand are crucial for the clustering of the receptor and the generation of the "endocytic synapsis." These steps seem to be important for tuning the strength of the signaling and determining the type of immunological response induced [58].

MGL oligomerization appears to occur independently by the structure of its ligand; however signaling and phenotypic changes can be different. It has shown that heterodimerization with other receptors and association with distinct PPRs modulate and enhance pathogen sensor function of several CLRs. MGL has been shown to synergize with TLR2-induced pathways in a study employing artificial model ligands, not present in the physiological microenvironment [59]. The working hypothesis that is sketched out by these evidences is that the ligand structure is not an absolute requirement for MGL oligomerization since this event appears to be independent of the nature of the ligand (peptide, protein, or single carbohydrate). However the structure becomes relevant for the functional outcome. The recruitment of alternative array of adaptor molecules at the "endocytic synapsis" may be involved in the differential intracellular sorting of the ligand and in the tuning of the signaling pathways crucial for DC polarization.

For this reason, it will be interesting to further characterize the interaction between MGL and its ligand, in terms of affinity, avidity, and ligand structure in order to design molecules to be employed as immunomodulators in therapeutic strategies for several pathologies, as well as for cancer.

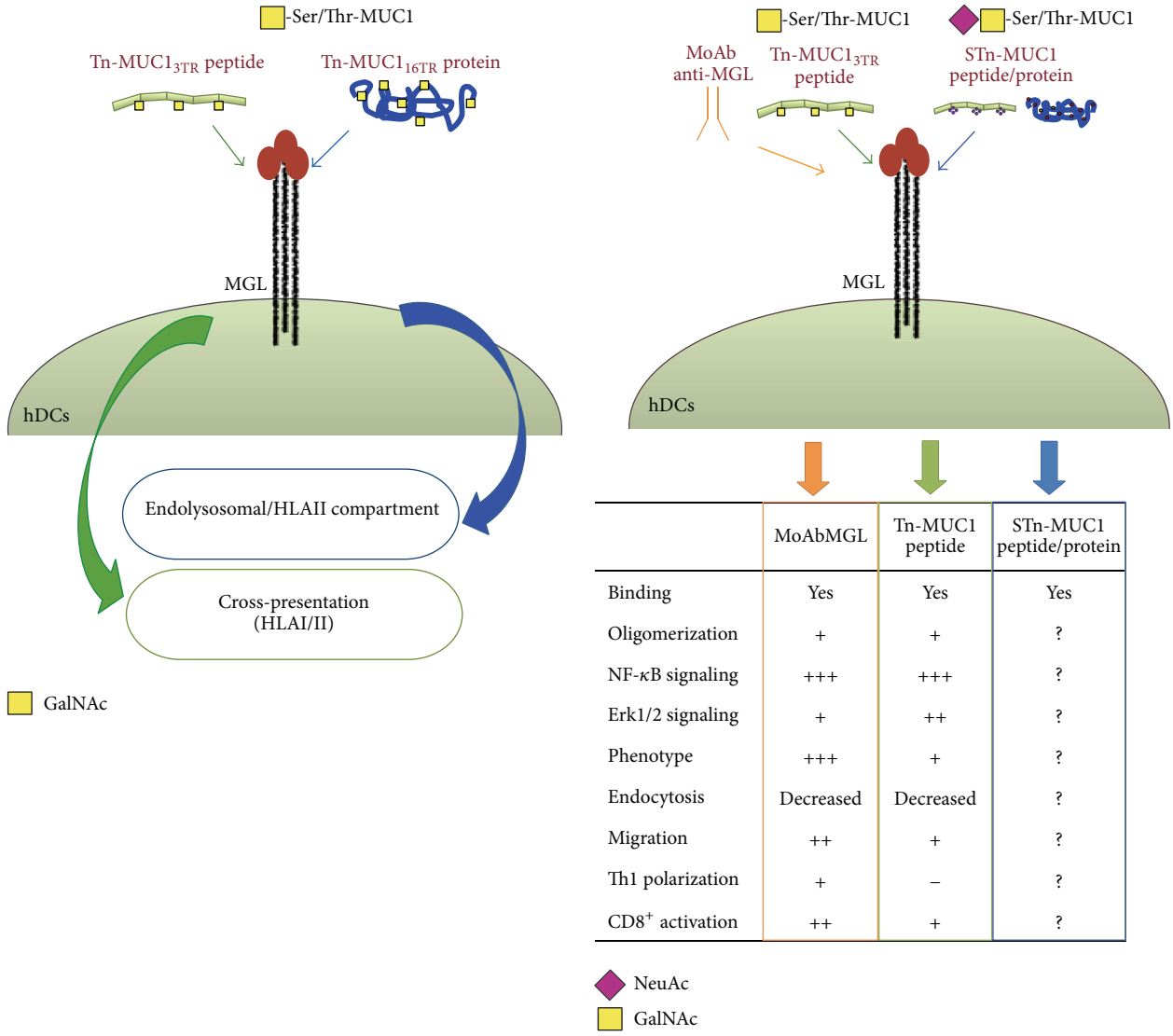
5. Conclusions

The study of MGL and its role in DCs as well as in macrophage functions is an open interesting area of research. The specificity for terminal GalNAc residues combined with the restricted tissue expression of this carbohydrate residue makes MGL a very specific detector of pathogens and a highly sensitive modulator of APCs in physiological as well as in inflammatory and cancer microenvironment.

The engagement of MGL by GalNAc carrying structures induces oligomerization of the receptor and internalization of the ligand; however the intracellular signaling pathways triggered can profoundly vary depending on the structure of the ligands, differentially affecting function of DCs and the resulting immune response.

In this view, the possibility to exploit MGL targeting as a way to modulate APC functions is an appealing hypothesis for the design of immunotherapeutic intervention.

Avidity and affinity of MGL ligand can be modulated by modifying Tn-density, the length, and steric structure of the backbone peptides. For cancer therapy, activation of DCs could be obtained by mimicking the effects of a danger signal and achieving an efficient presentation in HLA classes I and II pathways. Moreover, a wide variety of immunogens carrying Tn-epitopes could be envisaged, where the Tn-peptide stretch could only be a way to deliver other TAAs as well as other molecules. On the other hand, the fine-tuning of MGL function could lead to the induction of "Th2 oriented DCs" and interfere in the immunosuppressive/inflammatory network induced by pathogens infection. So far, investigators have focused their attention on the understanding the effect of MGL engagement on DCs. However, one has to bear in mind that the MGL-GalNAc ligand engagement mediates a one to one interaction between two cells. Thus functional changes may also occur in the cell carrying the GalNAc moieties. This mechanism seems to be particularly relevant in the physiological tissue homeostasis such as in T cell compartment. In this prospective, the design of optimal immunotherapeutic molecules based on MGL triggering could be novel approaches for the treatment of autoimmune diseases.



	MoAbMGL	Tn-MUC1 peptide	STn-MUC1 peptide/protein
Binding	Yes	Yes	Yes
Oligomerization	+	+	?
NF-κB signaling	+++	+++	?
Erk1/2 signaling	+	++	?
Phenotype	+++	+	?
Endocytosis	Decreased	Decreased	?
Migration	++	+	?
Th1 polarization	+	–	?
$CD8^+$ activation	++	+	?

Figure 3: The structure of tumor antigen formulations internalized by MGL influences their processing and DC performance. (a) Recombinant Tn-MUC1$_{16TR}$ protein remains blocked in DCs HLA class II compartment after internalization through MGL, while the shorter Tn-MUC1$_{3TR}$ peptide (60 amino acids) is processed in HLA classes I and II compartments. (b) Effects induced on DC functions by MGL engagement with MoAb anti-MGL and Tn-MUC1 peptide.

It is also important to retain that the molecular targeting and the choice of the antigen represent only "one side of the coin" in designing immunotherapeutic approaches. The choice of the optimal DC subset for priming T cells and strategies to contain or eliminate immunosuppression are other crucial parameters that should be considered to obtain an efficacious and long-lasting immune response.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Marianna Nuti and Aurelia Rughetti contributed equally to this work.

Acknowledgments

This work was supported by Ricerche Universitarie Sapienza (Chiara Napoletano: Ateneo 2014, C26A14WW3; Avvio alla Ricerca 2014: Ilaria Grazia Zizzari C26N14AHKZ; Salvatore Caponnetto C26N14X3H8) and by Associazione Italiana per la Ricerca sul Cancro (Marianna Nuti: AIRC 2015 cod.17432).

References

[1] B. Pulendran, K. Palucka, and J. Banchereau, "Sensing pathogens and tuning immune responses," *Science*, vol. 293, no. 5528, pp. 253–256, 2001.

[2] C. A. Janeway Jr. and R. Medzhitov, "Innate immune recognition," *Annual Review of Immunology*, vol. 20, pp. 197–216, 2002.

[3] I. M. Dambuza and G. D. Brown, "C-type lectins in immunity: recent developments," *Current Opinion in Immunology*, vol. 32, pp. 21–27, 2015.

[4] T. Kawai and S. Akira, "Toll-like receptor and RIG-1-like receptor signaling," *Annals of the New York Academy of Sciences*, vol. 1143, pp. 1–20, 2008.

[5] A. N. Zelensky and J. E. Gready, "The C-type lectin-like domain superfamily," *The FEBS Journal*, vol. 272, no. 24, pp. 6179–6217, 2005.

[6] Y. Van Kooyk and T. B. H. Geijtenbeek, "DC-SIGN: escape mechanism for pathogens," *Nature Reviews Immunology*, vol. 3, no. 9, pp. 697–709, 2003.

[7] A. Engering, T. B. H. Geijtenbeek, S. J. Van Vliet et al., "The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells," *The Journal of Immunology*, vol. 168, no. 5, pp. 2118–2126, 2002.

[8] T. B. H. Geijtenbeek and S. I. Gringhuis, "Signalling through C-type lectin receptors: shaping immune responses," *Nature Reviews Immunology*, vol. 9, no. 7, pp. 465–479, 2009.

[9] S. J. van Vliet, J. J. García-Vallejo, and Y. van Kooyk, "Dendritic cells and C-type lectin receptors: coupling innate to adaptive immune responses," *Immunology and Cell Biology*, vol. 86, no. 7, pp. 580–587, 2008.

[10] G. D. Brown, J. Herre, D. L. Williams, J. A. Willment, A. S. J. Marshall, and S. Gordon, "Dectin-1 mediates the biological effects of β-glucans," *Journal of Experimental Medicine*, vol. 197, no. 9, pp. 1119–1124, 2003.

[11] B. N. Gantner, R. M. Simmons, S. J. Canavera, S. Akira, and D. M. Underhill, "Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2," *Journal of Experimental Medicine*, vol. 197, no. 9, pp. 1107–1117, 2003.

[12] P. Chakraborty, D. Ghosh, and M. K. Basu, "Modulation of macrophage mannose receptor affects the uptake of virulent and avirulent *Leishmania donovani* promastigotes," *Journal of Parasitology*, vol. 87, no. 5, pp. 1023–1027, 2001.

[13] S. Oo-puthinan, K. Maenuma, M. Sakakura et al., "The amino acids involved in the distinct carbohydrate specificities between macrophage galactose-type C-type lectins 1 and 2 (CD301a and b) of mice," *Biochimica et Biophysica Acta (BBA): General Subjects*, vol. 1780, no. 2, pp. 89–100, 2008.

[14] L. A. J. O'Neill, "When signaling pathways collide: positive and negative regulation of toll-like receptor signal transduction," *Immunity*, vol. 29, no. 1, pp. 12–20, 2008.

[15] S. J. van Vliet, E. Saeland, and Y. van Kooyk, "Sweet preferences of MGL: carbohydrate specificity and function," *Trends in Immunology*, vol. 29, no. 2, pp. 83–90, 2008.

[16] C. Napoletano, A. Rughetti, M. P. Agervig Tarp et al., "Tumor-associated Tn-MUC1 glycoform is internalized through the macrophage galactose-type C-type lectin and delivered to the HLA class I and II compartments in dendritic cells," *Cancer Research*, vol. 67, no. 17, pp. 8358–8367, 2007.

[17] P. J. Tacken, I. J. M. de Vries, K. Gijzen et al., "Effective induction of naive and recall T-cell responses by targeting antigen to human dendritic cells via a humanized anti-DC-SIGN antibody," *Blood*, vol. 106, no. 4, pp. 1278–1285, 2005.

[18] G. Raes, L. Brys, B. K. Dahal et al., "Macrophage galactose-type C-type lectins as novel markers for alternatively activated macrophages elicited by parasitic infections and allergic airway inflammation," *Journal of Leukocyte Biology*, vol. 77, no. 3, pp. 321–327, 2005.

[19] S. J. Van Vliet, L. C. Paessens, V. C. M. Broks-van Den Berg, T. B. H. Geijtenbeek, and Y. Van Kooyk, "The C-type lectin macrophage galactose-type lectin impedes migration of immature APCs," *The Journal of Immunology*, vol. 181, no. 5, pp. 3148–3155, 2008.

[20] S. J. Van Vliet, E. Van Liempt, E. Saeland et al., "Carbohydrate profiling reveals a distinctive role for the C-type lectin MGL in the recognition of helminth parasites and tumor antigens by dendritic cells," *International Immunology*, vol. 17, no. 5, pp. 661–669, 2005.

[21] N. Mortezai, H. N. Behnken, A.-K. Kurze et al., "Tumor-associated Neu5Ac-Tn and Neu5Gc-Tn antigens bind to C-type lectin CLEC10A (CD301, MGL)," *Glycobiology*, vol. 23, no. 7, pp. 844–852, 2013.

[22] M. Tsuiji, M. Fujimori, Y. Ohashi et al., "Molecular cloning and characterization of a novel mouse macrophage C-type lectin, mMGL2, which has a distinct carbohydrate specificity from mMGL1," *The Journal of Biological Chemistry*, vol. 277, no. 32, pp. 28892–28901, 2002.

[23] Y. Kumamoto, K. Denda-Nagai, S. Aida, N. Higashi, and T. Irimura, "MGL2$^+$ dermal dendritic cells are sufficient to initiate contact hypersensitivity in vivo," *PLoS ONE*, vol. 4, no. 5, Article ID e5619, 2009.

[24] S. K. Singh, I. Streng-Ouwehand, M. Litjens et al., "Characterization of murine MGL1 and MGL2 C-type lectins: distinct glycan specificities and tumor binding properties," *Molecular Immunology*, vol. 46, no. 6, pp. 1240–1249, 2009.

[25] S. J. Van Vliet, C. A. Aarnoudse, V. C. M. Broks-van den Berg, M. Boks, T. B. H. Geijtenbeek, and Y. van Kooyk, "MGL-mediated internalization and antigen presentation by dendritic cells: a role for tyrosine-5," *European Journal of Immunology*, vol. 37, no. 8, pp. 2075–2081, 2007.

[26] N. M. van Sorge, N. M. C. Bleumink, S. J. van Vliet et al., "*N*-glycosylated proteins and distinct lipooligosaccharide glycoforms of Campylobacter jejuni target the human C-type lectin receptor MGL," *Cellular Microbiology*, vol. 11, no. 12, pp. 1768–1781, 2009.

[27] S. J. Van Vliet, L. Steeghs, S. C. M. Bruijns et al., "Variation of *Neisseria gonorrhoeae* lipooligosaccharide directs dendritic cell-induced T helper responses," *PLoS Pathogens*, vol. 5, no. 10, Article ID e1000625, 2009.

[28] S. J. Van Vliet, S. I. Gringhuis, T. B. H. Geijtenbeek, and Y. Van Kooyk, "Regulation of effector T cells by antigen-presenting cells via interaction of the C-type lectin MGL with CD45," *Nature Immunology*, vol. 7, no. 11, pp. 1200–1208, 2006.

[29] E. Saeland, S. J. Van Vliet, M. Bäckström et al., "The C-type lectin MGL expressed by dendritic cells detects glycan changes on MUC1 in colon carcinoma," *Cancer Immunology, Immunotherapy*, vol. 56, no. 8, pp. 1225–1236, 2007.

[30] I. G. Zizzari, P. Martufi, F. Battisti et al., "The macrophage galactose-type C-type lectin (MGL) modulates regulatory T cell functions," *PLoS ONE*, vol. 10, no. 7, Article ID e0132617, 2015.

[31] A. Takada, K. Fujioka, M. Tsuiji et al., "Human macrophage C-type lectin specific for galactose and N-acetylgalactosamine promotes filovirus entry," *Journal of Virology*, vol. 78, no. 6, pp. 2943–2947, 2004.

[32] E. Van Liempt, S. J. Van Vliet, A. Engering et al., "Schistosoma mansoni soluble egg antigens are internalized by human dendritic cells through multiple C-type lectins and suppress TLR-induced dendritic cell activation," *Molecular Immunology*, vol. 44, no. 10, pp. 2605–2615, 2007.

[33] M. H. J. Meevissen, N. N. Driessen, H. H. Smits et al., "Specific glycan elements determine differential binding of individual egg glycoproteins of the human parasite *Schistosoma mansoni* by host C-type lectin receptors," *International Journal for Parasitology*, vol. 42, no. 3, pp. 269–277, 2012.

[34] A. S. MacDonald, A. D. Straw, B. Bauman, and E. J. Pearce, "CD8- dendritic cell activation status plays an integral role in influencing Th2 response development," *The Journal of Immunology*, vol. 167, no. 4, pp. 1982–1988, 2001.

[35] E. C. De Jong, P. L. Vieira, P. Kalinski et al., "Microbial compounds selectively induce Th1 cell-promoting or Th2 cell-promoting dendritic cells in vitro with diverse th cell-polarizing signals," *The Journal of Immunology*, vol. 168, no. 4, pp. 1704–1709, 2002.

[36] C. M. Kane, L. Cervi, J. Sun et al., "Helminth antigens modulate TLR-initiated dendritic cell activation," *The Journal of Immunology*, vol. 173, no. 12, pp. 7454–7461, 2004.

[37] L. Cervi, A. S. MacDonald, C. Kane, F. Dzierszinski, and E. J. Pearce, "Cutting edge: dendritic cells copulsed with microbial and helminth antigens undergo modified maturation, segregate the antigens to distinct intracellular compartments, and concurrently induce microbe-specific Th1 and helminth-specific Th2 responses," *Journal of Immunology*, vol. 172, no. 4, pp. 2016–2020, 2004.

[38] D. Jankovic, M. C. Kullberg, P. Caspar, and A. Sher, "Parasite-induced Th2 polarization is associated with down-regulated dendritic cell responsiveness to Th1 stimuli and a transient delay in T lymphocyte cycling," *The Journal of Immunology*, vol. 173, no. 4, pp. 2419–2427, 2004.

[39] E. J. Klaver, L. M. Kuijk, L. C. Laan et al., "*Trichuris suis*-induced modulation of human dendritic cell function is glycan-mediated," *International Journal for Parasitology*, vol. 43, no. 3-4, pp. 191–200, 2013.

[40] S. J. Jenkins, G. Perona-Wright, A. G. F. Worsley, N. Ishii, and A. S. MacDonald, "Dendritic cell expression of OX40 ligand acts as a costimulatory, not polarizing, signal for optimal Th2 priming and memory induction in vivo," *Journal of Immunology*, vol. 179, no. 6, pp. 3515–3523, 2007.

[41] M. Bruckner, D. Dickel, E. Singer, and D. F. Legler, "Converse regulation of CCR7-driven human dendritic cell migration by prostaglandin E_2 and liver X receptor activation," *European Journal of Immunology*, vol. 42, no. 11, pp. 2949–2958, 2012.

[42] I. G. Zizzari, C. Napoletano, A. Rughetti, H. Rahimi, and M. Nuti, "Seasonal modulation of the C-type lectin MGL on human DCs," *Open Journal of Immunology*, vol. 3, no. 4, pp. 218–220, 2013.

[43] S. J. Van Vliet, E. Van Liempt, T. B. H. Geijtenbeek, and Y. Van Kooyk, "Differential regulation of C-type lectin expression on tolerogenic dendritic cell subsets," *Immunobiology*, vol. 211, no. 6–8, pp. 577–585, 2006.

[44] D. Li, G. Romain, A.-L. Flamar et al., "Targeting self- and foreign antigens to dendritic cells via DC-ASGPR generates IL-10-producing suppressive $CD4^+$ T cells," *Journal of Experimental Medicine*, vol. 209, no. 1, pp. 109–121, 2012.

[45] J. M. Burchell, A. Mungul, and J. Taylor-Papadimitriou, "O-linked glycosylation in the mammary gland: changes that occur during malignancy," *Journal of Mammary Gland Biology and Neoplasia*, vol. 6, no. 3, pp. 355–364, 2001.

[46] C. Welinder, B. Baldetorp, C. Borrebaeck, B.-M. Fredlund, and B. Jansson, "A new murine IgG1 anti-Tn monoclonal antibody with in vivo anti-tumor activity," *Glycobiology*, vol. 21, no. 8, pp. 1097–1107, 2011.

[47] P. Nollau, G. Wolters-Eisfeld, N. Mortezai et al., "Protein domain histochemistry (PDH): binding of the carbohydrate recognition domain (CRD) of recombinant human glycoreceptor CLEC10A (CD301) to formalin-fixed, paraffin embedded breast cancer tissues," *Journal of Histochemistry and Cytochemistry*, vol. 61, no. 3, pp. 199–205, 2013.

[48] K. Lenos, J. A. Goos, I. M. Vuist et al., "MGL ligand expression is correlated to BRAF mutation and associated with poor survival of stage III colon cancer patients," *Oncotarget*, vol. 6, no. 28, pp. 26278–26290, 2015.

[49] S. H. Itzkowitz, M. Yuan, C. K. Montgomery et al., "Expression of Tn, sialosyl-Tn, and T antigens in human colon cancer," *Cancer Research*, vol. 49, no. 1, pp. 197–204, 1989.

[50] D. J. Gill, K. M. Tham, J. Chia et al., "Initiation of GalNAc-type O-glycosylation in the endoplasmic reticulum promotes cancer cell invasiveness," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 34, pp. E3152–E3161, 2013.

[51] T. Freire, R. Lo-Man, S. Bay, and C. Leclerc, "Tn glycosylation of the MUC6 protein modulates its immunogenicity and promotes the induction of Th17-biased T cell responses," *The Journal of Biological Chemistry*, vol. 286, no. 10, pp. 7797–7811, 2011.

[52] R. Beatson, G. Maurstad, G. Picco et al., "The breast cancer-associated glycoforms of MUC1, MUC1-Tn and sialyl-Tn, are expressed in *COSMC* wild-type cells and bind the C-type lectin MGL," *PLoS ONE*, vol. 10, no. 5, Article ID e0125994, 2015.

[53] S. A. Jégouzo, A. Quintero-Martínez, X. Ouyang, Á. Dos Santos, M. E. Taylor, and K. Drickamer, "Organization of the extracellular portion of the macrophage galactose receptor: a trimeric cluster of simple binding sites for *N*-acetylgalactosamine," *Glycobiology*, vol. 23, no. 7, pp. 853–864, 2013.

[54] D. W. Miles, L. C. Happerfield, P. Smith et al., "Expression of sialyl-Tn predicts the effect of adjuvant chemotherapy in node-positive breast cancer," *British Journal of Cancer*, vol. 70, no. 6, pp. 1272–1275, 1994.

[55] C. Napoletano, I. G. Zizzari, A. Rughetti et al., "Targeting of macrophage galactose-type C-type lectin (MGL) induces DC signaling and activation," *European Journal of Immunology*, vol. 42, no. 4, pp. 936–945, 2012.

[56] N. Higashi, K. Fujioka, K. Denda-Nagai et al., "The macrophage C-type lectin specific for galactose/*N*-acetylgalactosamine is an endocytic receptor expressed on monocyte-derived immature dendritic cells," *Journal of Biological Chemistry*, vol. 277, no. 23, pp. 20686–20693, 2002.

[57] A. Rughetti, H. Rahimi, F. Belleudi et al., "Microvesicle cargo of tumor-associated MUC1 to dendritic cells allows cross-presentation and specific carbohydrate processing," *Cancer Immunology Research*, vol. 2, no. 2, pp. 177–186, 2014.

[58] S. Iborra and D. Sancho, "Signalling versatility following self and non-self sensing by myeloid C-type lectin receptors," *Immunobiology*, vol. 220, no. 2, pp. 175–184, 2015.

[59] S. J. van Vliet, S. Bay, I. M. Vuist et al., "MGL signaling augments TLR2-mediated responses for enhanced IL-10 and TNF-α secretion," *Journal of Leukocyte Biology*, vol. 94, no. 2, pp. 315–323, 2013.

Hindawi Publishing Corporation
Journal of Immunology Research
Volume 2015, Article ID 168574, 13 pages
http://dx.doi.org/10.1155/2015/168574

Review Article

The Good, the Bad, and the Ugly of Dendritic Cells during Prion Disease

Neil Andrew Mabbott and Barry Matthew Bradford

The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush, Midlothian EH25 9RG, UK

Correspondence should be addressed to Neil Andrew Mabbott; neil.mabbott@roslin.ed.ac.uk

Received 29 September 2015; Accepted 15 November 2015

Academic Editor: Silvia Beatriz Boscardin

Prions are a unique group of proteinaceous pathogens which cause neurodegenerative disease and can be transmitted by a variety of exposure routes. After peripheral exposure, the accumulation and replication of prions within secondary lymphoid organs are obligatory for their efficient spread from the periphery to the brain where they ultimately cause neurodegeneration and death. Mononuclear phagocytes (MNP) are a heterogeneous population of dendritic cells (DC) and macrophages. These cells are abundant throughout the body and display a diverse range of roles based on their anatomical locations. For example, some MNP are strategically situated to provide a first line of defence against pathogens by phagocytosing and destroying them. Conventional DC are potent antigen presenting cells and migrate via the lymphatics to the draining lymphoid tissue where they present the antigens to lymphocytes. The diverse roles of MNP are also reflected in various ways in which they interact with prions and in doing so impact on disease pathogenesis. Indeed, some studies suggest that prions exploit conventional DC to infect the host. Here we review our current understanding of the influence of MNP in the pathogenesis of the acquired prion diseases with particular emphasis on the role of conventional DC.

1. Introduction

Prion diseases, or transmissible spongiform encephalopathies, are subacute neurodegenerative diseases affecting humans and certain domestic and free-ranging animal species. These diseases are characterized by the presence of aggregations of PrP^{Sc}, abnormally folded isoforms of the cellular prion protein (PrP^{C}), in affected tissues. Although the precise nature of the infectious prion is still the subject of intense debate, prion infectivity copurifies with PrP^{Sc} which is considered to constitute the major component of the infectious agent [1, 2]. The accumulation of PrP^{Sc} in the central nervous system (CNS) of prion-infected hosts is accompanied by neuronal loss, spongiosis, and reactive glial responses (Figure 1). Some prion diseases appear to have idiopathic aetiology. These may arise spontaneously within the CNS (such as sporadic Creutzfeldt-Jakob disease (CJD)) or are associated with polymorphisms within the *PRNP* gene (which encodes PrP^{C}) which some consider predisposes the prion protein to abnormally fold into the disease-specific isoform (such as Gerstmann-Straussler-Scheinker syndrome). Many other prion diseases, including natural sheep scrapie, bovine spongiform encephalopathy, and chronic wasting disease in cervids and kuru and variant Creutzfeldt-Jakob disease (vCJD) in humans, are acquired following exposure to prions, for example, by oral consumption of prion-contaminated food. For the efficient transmission of prions to the CNS after peripheral exposure (a process termed *neuroinvasion*), the replication of prions within secondary lymphoid tissues is crucial [3]. Within lymphoid tissues, prions replicate upon stromal-derived follicular dendritic cells (FDC) located within the B cell follicles [4–6] (Figure 2). Following their replication and amplification upon FDC, the prions subsequently spread along neurones of the sympathetic and parasympathetic nervous systems, accessing the CNS wherein they ultimately cause neurodegeneration resulting in the death of the host [7–10].

Mononuclear phagocytes (MNP) arise from haematopoietic precursor cells within the bone marrow and are a heterogeneous population of monocytes, macrophages, and

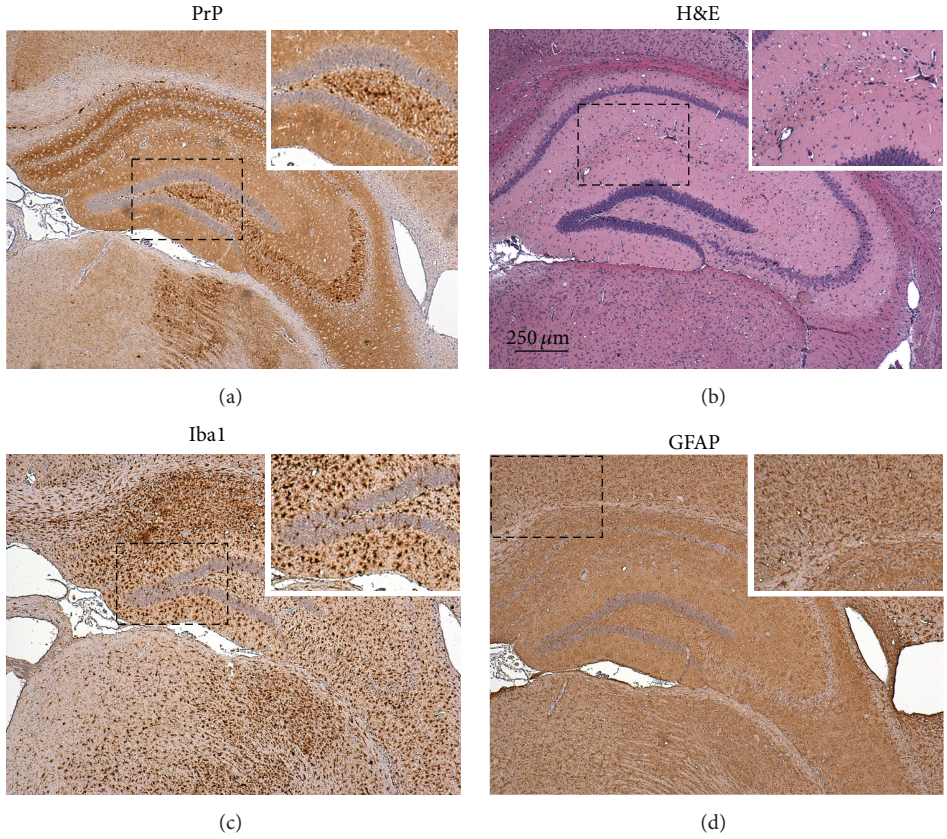


Figure 1: Neuropathological characteristics of prion disease within the brains of clinically affected mice. (a) Prion diseases are characterized by the presence of aggregations of abnormally folded, disease-specific prion protein (PrP) in affected tissues (brown). In the brain, as shown here, these accumulations are accompanied by extensive neuronal loss, spongiform change (indicated by vacuolation in panel "(b)"), reactive microglia (Iba1$^+$ cells, panel "(c)," brown), and reactive astrocytes expressing high levels of glial fibrillary acidic protein (GFAP, panel "(d)," brown). Sections are counterstained with haematoxylin (blue).

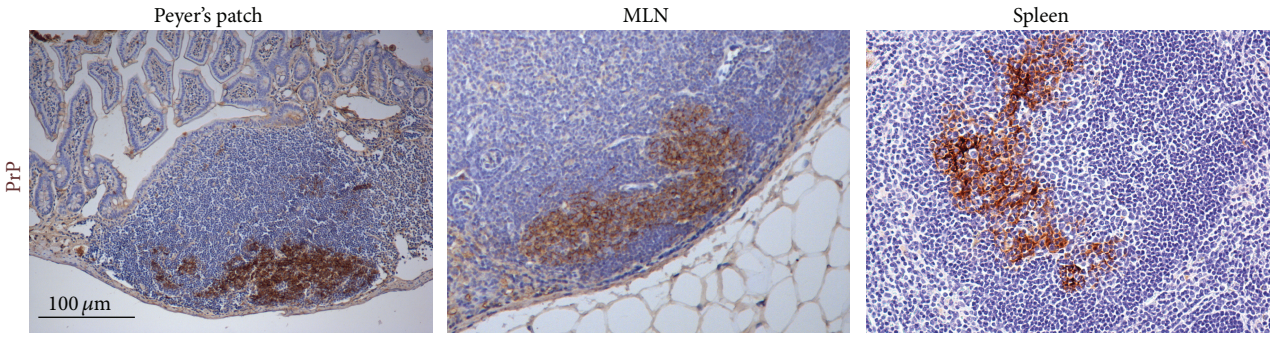


Figure 2: Stromal-derived follicular dendritic cells are important sites of prion accumulation and replication in the B cell follicles of secondary lymphoid tissues. Detection of high levels of abnormally folded, disease-specific prion protein (PrP, brown) in Peyer's patches, mesenteric lymph nodes (MLN), and spleen of a mouse infected with ME7 scrapie prions. Sections are counterstained with haematoxylin (blue).

dendritic cells [11–13]. These cells are abundant throughout the body and possess a diverse range of functions based on the anatomic locations they occupy. For example, some MNP are strategically situated at exposure sites such as the skin or intestinal lamina propria to provide a first line of defence against pathogens by phagocytosing and destroying them in their phagolysosomal compartments. Others, such as conventional dendritic cells (DC), are potent antigen presenting cells and provide an important link between the innate and adaptive immune systems. These MNP are located to efficiently sample host tissues and fluids for pathogens and their antigens (Figure 3). The immature conventional DC at these sites are highly phagocytic. Following the uptake of pathogens or antigens, these cells typically undergo

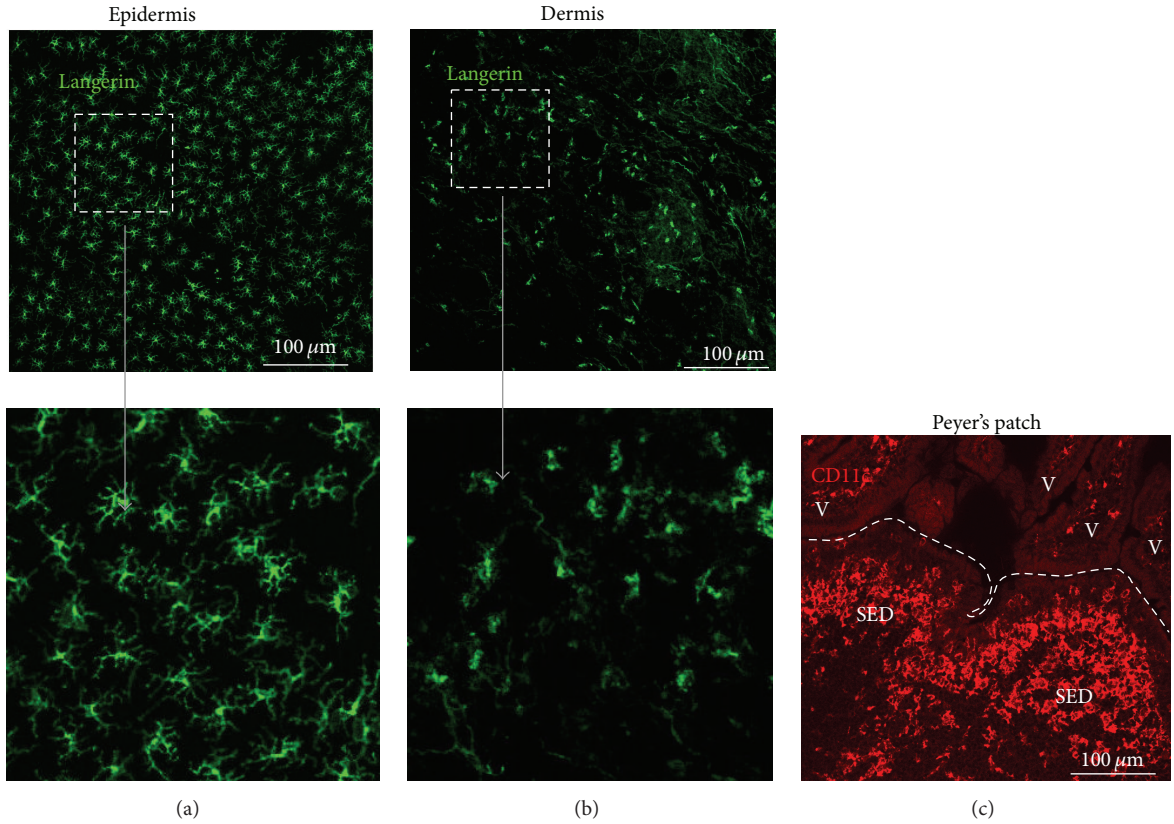


Figure 3: Mononuclear phagocytes (MNP) are a heterogeneous population of monocytes, macrophages, and dendritic cells and are abundant throughout the body. MNP are strategically situated at exposure sites such as in the epidermis or dermis of the skin (panels "(a)" and "(b)," resp.) and in the intestinal lamina propria where they provide a first line of defence against pathogens. (a and b) Whole-mount immunohistochemical detection of langerin$^+$ Langerhans cells in the epidermis (green, panels "(a)") and langerin$^+$ conventional DC in the dermis (green, panels "(b)"). The boxed region in the upper panels is shown at higher magnification in the adjacent lower panels. (c) CD11c$^+$ MNP (red) are abundant in Peyer's patches and the intestinal lamina propria. SED, subepithelial dome region on Peyer's patch; V, villus; broken line indicates the boundary of the epithelium overlying Peyer's patch.

maturation and migrate via the lymphatics to the draining (regional) lymphoid tissue, such as the mesenteric lymph nodes (MLN) associated with the intestine [14], where they present the antigens to lymphocytes to initiate an antigen-specific (adaptive) immune response or induce tolerance [15]. Other MNP populations appear to play an important role within lymphoid tissues in the transfer of intact antigens to B cells [16, 17]. In this review, it is important to remember that the migratory, bone marrow-derived conventional DC [15] are entirely distinct from the stromal derived FDC [18, 19] which have been shown to be the critical sites of prion replication in lymphoid tissues [6]. The FDC, in contrast, are localized within B cell follicles of lymphoid tissues, derive from ubiquitous perivascular precursor cells [19], are tissue fixed and nonphagocytic. In contrast to conventional DC, FDC are long-lived cells which can retain native antigens on their surfaces for long periods.

Viable commensal bacteria can be recovered from DC migrating from the intestine [20] and some pathogenic microorganisms may exploit DC as an efficient way to infect host tissues [21]. In the transient absence of CD11c$^+$ DC at the time of peripheral exposure, the early accumulation of prions in the draining lymphoid tissue was blocked and disease susceptibility reduced [22–24]. These data imply that prions may also exploit conventional DC to infect the host. Thus, in this review we discuss our current understanding of the role of MNP in the pathogenesis of the acquired prion diseases with particular emphasis on conventional DC.

2. Conventional DC: A Multifunctional Cellular Component of the Innate Immune System

MNP such as conventional DC exhibit a diverse array of functions in the mammalian innate immune system. This is reflected in the various ways in which they may interact with prions, and, by doing so, impact on prion disease pathogenesis (Figure 4). Following their uptake by conventional DC, the prions may (i) activate innate immune responses and be sequestered and partially degraded within the cell's phagolysosomal compartments; (ii) undergo amplification (replication) since these cells express the substrate PrP^C; (iii) activate acquired immune responses and induce a specific

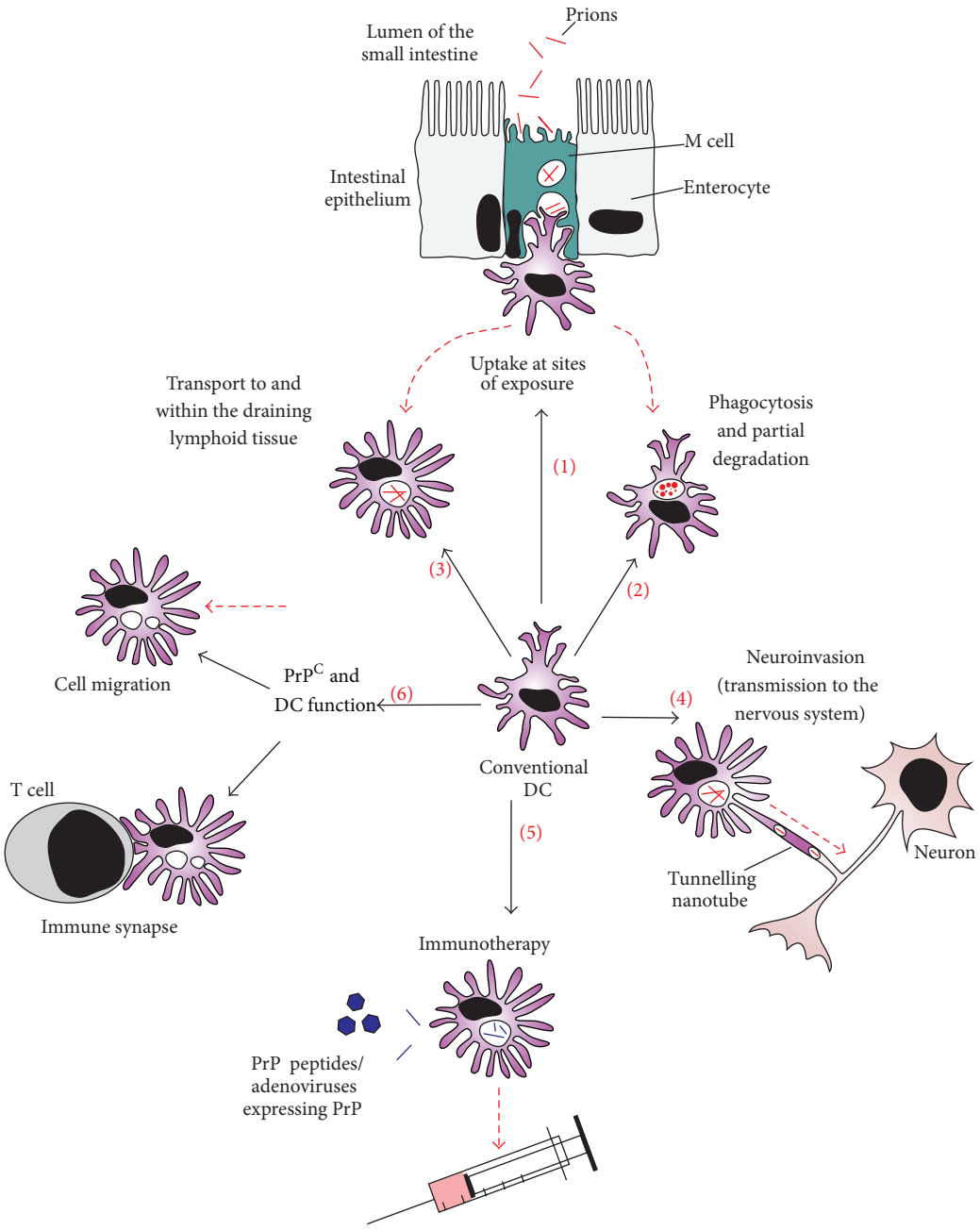


Figure 4: The influence of DC on prion disease pathogenesis. (1) Conventional DC are strategically placed throughout the mammalian host and are amongst the first cell populations to interact with prions. Following their uptake of prions DC have been proposed to exert a diverse range of contrasting effects on prion disease pathogenesis which may have a significant outcome on the spread of infection to the CNS. (2) Some studies have suggested that DC may help to protect the host against infection by attempting to sequester and destroy the prions [25–29]. (3) Others suggest that prions may exploit the migratory characteristics of DC to facilitate their efficient propagation from the site of exposure to the lymphoid tissues [22–24, 34, 39]. (4) DC may also play an important role in the subsequent transfer of prions to the CNS by bridging the gap between the immune and peripheral nervous systems [36, 61, 91–93]. (5) The adoptive transfer of PrP peptide-loaded DC into mice can overcome host tolerance towards PrP and prolong survival time after prion infection. This implies that DC could be manipulated to provide immunotherapeutic protection against prion diseases [54, 106, 107]. (6) The physiological function of cellular PrP^C is uncertain but in DC may play a role in the immune synapse or in the regulation of cell migration [51, 52].

immune response to prions as conventional DC are potent antigen-presenting cells; (iv) be conveyed from the site of exposure to sites of prion replication within the draining lymphoid tissue. The sections below describe the many studies which have attempted to address the potential contribution of these roles to prion disease pathogenesis.

3. *In Vitro* Cultivated DC Can Acquire and Destroy Prions

"Immature" conventional DC are highly phagocytic cells and have the potential to sequester and destroy prions in a similar manner to that in which they process peptide antigens for presentation to T cells in association with MHC class II. Data from several independent studies support this hypothesis and have shown that *in vitro* cultivated DC-like cells can readily acquire and degrade prions [25–28]. Within these cells, the prion-specific PrP^{Sc} appears to be preferentially degraded by cysteine proteases [29]. These data are congruent with data from similar studies using macrophages which show they can also acquire and degrade prions after extended *in vitro* exposure [30, 31]. Whether these data accurately reflect the handling and processing of prions by conventional DC *in vivo* is uncertain since these cells can retain high levels of infectious prions in infected rodents [32–36]. Furthermore, when macrophages are depleted *in vivo* in prion-infected hosts, higher concentrations of prions are recovered from their lymphoid tissues [37, 38]. In contrast, depletion of $CD11c^{+}$ cells impedes the early accumulation of prions in the draining lymphoid tissue [22–24, 39] (see below).

4. DC Are Not Important Sites of Prion Replication

Although conventional DC are typically considered to internalize antigens which they then process into short peptides and present them on their surfaces to T cells, some MNP populations including certain conventional DC subsets appear to be equipped with both degradative and nondegradative antigen handling pathways [40, 41]. These distinct pathways may enable conventional DC to present processed peptide antigens to T cells or native antigens to B cells. During prion infection DC can sequester high levels of prions [32–36], but these cells are highly unlikely to be acting as important early sites of prion replication or amplification. Expression of the cellular prion protein, PrP^{C}, is obligatory for prion replication, and MNP including conventional DC in mice, humans, and cattle express PrP^{C} on their surfaces [42–44]. However, several studies have shown that prion replication within the secondary lymphoid tissues and disease pathogenesis are not influenced by the absence of PrP^{C} expression in haematopoietic cells [6, 45–48]. Thus, the role of DC during prion pathogenesis is more complex than simply acting as sites of prion replication.

5. The Enigmatic Function of PrP^{C} in the Immune System

The cellular prion protein, PrP^{C}, is 30–35 kDa glycoprotein linked to the cell surface via a glycosylphosphatidylinositol anchor. The precise function of PrP^{C} in mammalian cells remains elusive, but the expression of PrP^{C} by many immune cell populations, including conventional DC, implies a role in immune function [42–44]. However, mice that lack PrP^{C} expression in the haematopoietic compartment display no obvious immune deficit and are able to maintain antigen-specific antibody responses and affinity maturation [49]. Some studies have suggested that PrP^{C} may regulate phagocytosis. Upon further scrutiny, a separate study revealed that the reduced ability of MNP to phagocytose apoptotic cells in $Prnp^{-/-}$ mice was due to effects on a linked locus encoding the signal regulatory protein α (*Sirpa*) gene rather than the absence of PrP^{C} expression [50]. Microscopical analyses show PrP^{C} accumulates at contact sites between T cells and antigen-loaded conventional DC implying a role in the immune synapse between these cell populations. Consistent with this, the absence of PrP^{C} in antigen-presenting cells impacted on their ability to stimulate T cells [51].

A separate study has proposed that PrP^{C} may regulate human monocyte migration by modulating cell adhesion dynamics [52]. The authors propose that PrP^{C} regulates β1-integrin-mediated adhesion by modulating the remodelling of the actin cytoskeleton through the RhoA-cofilin pathway.

6. Induction of Specific Immunity against Prions

Although conventional DC are potent antigen-presenting cells and play an important role in the induction of antigen-specific immune responses, these cells are unlikely to play a role in the induction of specific immunity against prions. The prion protein is tolerated by the host immune system due to the widespread expression of PrP^{C} throughout the body. This prevents the induction of specific cell-mediated and antibody-mediated immune responses to PrP^{Sc}, the major component of infectious prions [53]. Despite this, a cell-based immunotherapy approach may be possible against prions as experiments have shown that the adoptive transfer of PrP peptide-loaded conventional DC into mice can overcome host tolerance towards PrP and prolong survival time after peripheral prion exposure [54].

7. DC and the Propagation of Prions to Draining Lymphoid Tissues

Some DC populations have been shown to have the ability to capture and retain unprocessed antigens in their native states and transfer them intactly to naïve B cells to initiate a specific antibody response [17]. Viable commensal bacteria can also be recovered from conventional DC migrating from the intestine [20]. The demonstration that some pathogenic

microorganisms appear to exploit migratory DC to enable their delivery to lymphoid tissues [21, 55–57] raised the hypothesis that DC may play a similar role in the initial delivery of prions from the site of infection (such as the gut lumen) to the draining lymphoid tissues (such as the gut-associated lymphoid tissues after oral exposure). This hypothesis was further supported by the observation that some migrating intestinal DC in the afferent mesenteric lymph had acquired PrP^{Sc} following its injection into the gut lumen [34]. Subsequent studies have since shown that, in the absence of migratory DC at the time of peripheral exposure, the early accumulation of prions in the draining lymphoid tissue and the subsequent spread of disease to the CNS are both impeded [22–24, 39]. However, not all DC subsets appear to share this property. For example, whereas the depletion of $CD11c^+$ cells (using CD11c-DTR-eGFP-tg mice) dramatically impedes oral prion pathogenesis [22], specific depletion of $CD8^+CD11c^+$ cells (using CD11c-N17Rac1-tg mice) does not [24]. Similarly, prion pathogenesis following infection via skin lesions was impaired in the specific absence of $CD11c^+$ $langerin^-$ dermal DC but was not affected in the absence of epidermal Langerhans cells or $langerin^+$ dermal DC [39].

Chemokines help to attract lymphocytes and DC to lymphoid tissues and control their positioning within them. For example, the chemokines CCL19 and CCL21 are constitutively expressed by stromal cells within the T cell zones and mediate the homing of chemokine receptor CCR7-expressing naïve T cells and mature DC towards them [58]. The positioning of DC within the interfollicular T cell regions of Peyer's patches and their steady-state migration from Peyer's patches to the MLN are likewise dependent upon CCR7-CCL19/CCL21-signalling [59]. However, the CCL19/CCL21-mediated attraction of DC is unlikely to influence prion neuroinvasion from Peyer's patches since oral prion pathogenesis is unaffected in *plt* mice which lack CCL19 and CCL21 [33]. This observation is consistent with data from other studies showing that Peyer's patches in the small intestine, not the MLN which collect the lymph and cells draining the intestine [14], are the critical sites of prion accumulation and neuroinvasion after oral prion exposure [3, 60]. Prion pathogenesis is likewise unaffected in the specific absence of T cells [61].

The demonstration that the accumulation of prions upon FDC in the draining lymphoid tissues was prevented in the absence of DC at the time of exposure [22–24, 39] implied that prions exploit these cells to access the draining lymphoid tissue, perhaps by using them as "Trojan horses." The detection of PrP^{Sc}-containing DC within the villous lacteals and submucosal lymphatics in the intestines of sheep soon after exposure to prions by oral infection or by injection into ligated gut loops implies a similar role [62–64]. Distinct DC subsets have been described that can transport native antigen to B cells *in vivo* [17, 65, 66]. The chemokine CXCL13 is highly expressed by FDC and follicular stromal cells in the B cell follicles of lymphoid tissues and modulates the homing of CXCR5-expressing B cells into them [67, 68]. The migration of certain populations of splenic DC and dermal DC into B cells follicles is also mediated by CXCL13-CXCR5 signalling [69, 70]. During virus infection, DC within the medullary sinus have been shown to capture lymph-borne influenza virus particles and subsequently migrate to the FDC-containing B cell follicles [71]. Further studies are clearly necessary to determine whether, after acquiring prions, DC similarly migrate similarly towards B cell follicles and in doing so infect FDC.

Although several studies suggest that DC may play an important role in the initial delivery of prions to and within the draining lymphoid tissues, the possibility that some of the prions may enter these tissues in a cell-free manner should not be excluded [39, 72, 73].

8. How Do DC Acquire Prions?

Whether DC acquire and endocytose prions via a specific receptor or receptors is uncertain, but the neurotoxic prion protein fragment $PrP_{106–126}$ is a chemoattractant for monocyte-derived DC [74]. Some MNP subsets express cellular PrP^C highly which may itself act as a receptor for prion-specific PrP^{Sc} [42–44]. However, if DC do acquire some prions in a PrP^C-dependent manner, it does not play a major role in disease pathogenesis. The propagation of prions from various peripheral sites of exposure to FDC and their subsequent neuroinvasion are not influenced by a lack of PrP^C expression by haematopoietic cells [6, 45–48]. These observations suggest the existence of other receptors on the surfaces of DC besides PrP^C that they may use to acquire prions.

The FDC within the B cell follicles are considered to acquire prions in the form of complement-opsonized complexes [75, 76]. Conventional DC may similarly indirectly acquire prions following their opsonisation by complement components such as C1q and C3 [72, 77]. The complement C1q-dependent uptake of prions by conventional DC appeared to be complement receptor- (CR-) mediated [77]. The identity of the specific receptor which mediates the uptake of complement-opsonized prions by conventional DC is uncertain, but many candidate molecules such as CR1 (CD35), CR2 (CD21), CR4 (CD11c/CD18), calreticulin, CD93, and SIGN-R1 (CD209b) are expressed by specific populations of these cells and can bind C1q [72, 77]. In other studies, it is interesting to note that the SIGN-R1-mediated uptake of influenza virus by DC lining the medullary sinus of lymph nodes stimulates their subsequent migration towards FDC [71]. After oral exposure, it is possible that the prions are acquired from the gut lumen in complex with dietary ferritin [78]. Finally, since MNP such as conventional DC are highly phagocytic, they may simply acquire prions nonspecifically as the cells constitutively sample their microenvironment, for example, via micropinocytosis.

9. Plasmacytoid DC also Sequester Prions

The plasmacytoid DC are a distinct subset of MNP which rapidly secrete large amounts of type I interferon (IFN-α/β) in response to foreign nucleic acids such as during virus infection [79]. One study has shown that plasmacytoid DC, like

conventional DC, can also sequester high levels of infectious prions during infection [36]. The consequences that this may have on prion disease pathogenesis are uncertain. Plasmacytoid DC are unlikely to play a role in the propagation of prions to the draining lymphoid tissues since these cells do not migrate in the lymphatics during the steady-state or following activation [80]. Plasmacytoid DC also express negligible levels of PrP^{C}, even after activation, so like classical DC they are unlikely to be important sources of prion replication [81]. Prion disease also does not induce the synthesis of significant levels of IFN [82–84], and treatment of mice soon after prion infection with polyriboinosinic-polyribocytidylic acid (poly(I:C)), which stimulates type I IFN production, does not alter disease pathogenesis [85, 86]. Splenic plasmacytoid DC may simply be attempting to sequester and destroy prions following their amplification by FDC. However, some studies have suggested that plasmacytoid DC and classical DC [36, 87] may play a role in prion neuroinvasion by facilitating the subsequent propagation of prions to peripheral nerves (see below).

10. DC and the Propagation of Prions between the Immune and Nervous Systems

Following their amplification upon FDC prions subsequently infect the peripheral nerves within the lymphoid tissue [8, 9, 88]. The prions then spread along the nerves of both the sympathetic and parasympathetic nervous systems and subsequently infect the CNS where they cause neurodegeneration leading to the death of the host [9, 10]. How prions spread between FDC and peripheral nerves is not known as these cells do not make significant physical contacts or synapses. Within peripheral tissues, there is much crosstalk between MNP and peripheral nerves. For example, in the intestine MNP/conventional DC are abundant in the muscular layer where they interact with enteric neurones and help regulate gastrointestinal motility [89, 90]. Given their migratory properties, experiments have sought to determine whether DC might also bridge the gap between FDC and peripheral nerves during prion disease.

Data from *in vitro* coculture studies show that prion-infected DC could potentially transfer prions to primary neurones or mouse neuroblastoma N2a cells [91–93]. Efficient prion transfer between these populations required cell-cell contact [92, 93]. Furthermore, when fixed bone marrow-derived DC were used, this activity was blocked implying an active process was required [92]. Data from a detailed *in vitro* study have proposed that tunnelling nanotubes (TNT), thin membrane-bound cylinders of cytoplasm which can connect neighbouring cells, might represent a novel method through which the intracellular exchange of prions between these cells may occur [91]. Within the TNT the PrP^{Sc} appears to travel in endolysosomal vesicles [94]. Whether significant transfer of prions between cells by TNT occurs in the dynamic environment of the lymphoid tissues *in vivo* remains to be determined. However, the analysis of lymphoid tissues from HIV patients shows intercellular transfer via a similar mechanism is possible. Xu and colleagues revealed that HIV-1-infected macrophages were able to establish long range intercellular connections (consistent with TNT) with B cells [95]. These intercellular conduits were exploited by the virus to deliver a virus encoded immunosuppressive factor to B cells to enable it to suppress the humoral response.

Prions have also been proposed to be released from infected cells in the form of small endosomal-derived vesicles termed exosomes [96]. Therefore, during prion infection DC may also release significant amounts of infectious prions in this manner and in doing so enhance their ability to infect neighbouring cells [36].

An *in vivo* study has suggested that prion-infected DC alone are potentially sufficient to transfer infection directly to the nervous system. Immunodeficient $Rag1^{-/-}$ mice lack T and B cells and are indirectly deficient in FDC. As a consequence these mice are refractory to peripheral infection with prions [61]. Despite this, live prion-infected DC were sufficient to transmit disease after intravenous injection into $Rag1^{-/-}$ mice [32]. Since these mice lack mature FDC and are unable to replicate prions in their lymphoid tissues, these data implied that the DC had transferred the prions directly to the peripheral nervous system. However, an independent study using FDC-deficient $Tnfr1^{-/-}$ mice was unable to demonstrate significant direct infection of the nervous system by prion-infected DC [35]. The precise reason for the discrepancy between these two studies is uncertain but may relate to the much higher density of peripheral nerves in the spleens of $Rag1^{-/-}$ mice when compared to $Tnfr1^{-/-}$ mice [35]. Clearly further studies are necessary to determine the precise contribution of DC and DC-derived tunnelling nanotubes or exosomes to the transfer of prions between FDC and peripheral nerves *in vivo*.

A subset of MNP with apparent conventional DC characteristics has been described in the mouse brain [97]. Within the brains of variant CJD patients deposits of PrP^{Sc} have been described in vascular-associated DC [98], and another study has proposed that $CD205^{+}$ (DEC-205) expressing DC may also migrate into the murine brain during prion disease [99]. Under certain circumstances, monocytes may also traffic to the brain and, in doing so, act as potential vectors for the delivery of pathogens such as virus or prions or misfolded aggregates of Alzheimer's disease-related amyloid β protein [100]. The possibility cannot therefore be entirely exclude that prion-infected conventional DC contribute to the establishment of prion infection in the CNS. Complement components C1q and C3 associate with PrP^{Sc} in the brains of prion-infected mice [101], raising the possibility that prions are acquired by DC in the CNS in a complement-dependent manner [72, 77].

The detection of prion-specific PrP within the circumventricular organs of the brain has been reported to be an early feature in scrapie-affected sheep [102]. Due to the presence of their fenestrated capillaries, the circumventricular organs are important sites of molecular exchange between the blood stream and the CNS. However, during prion disease monocytic infiltration into the circumventricular organs is

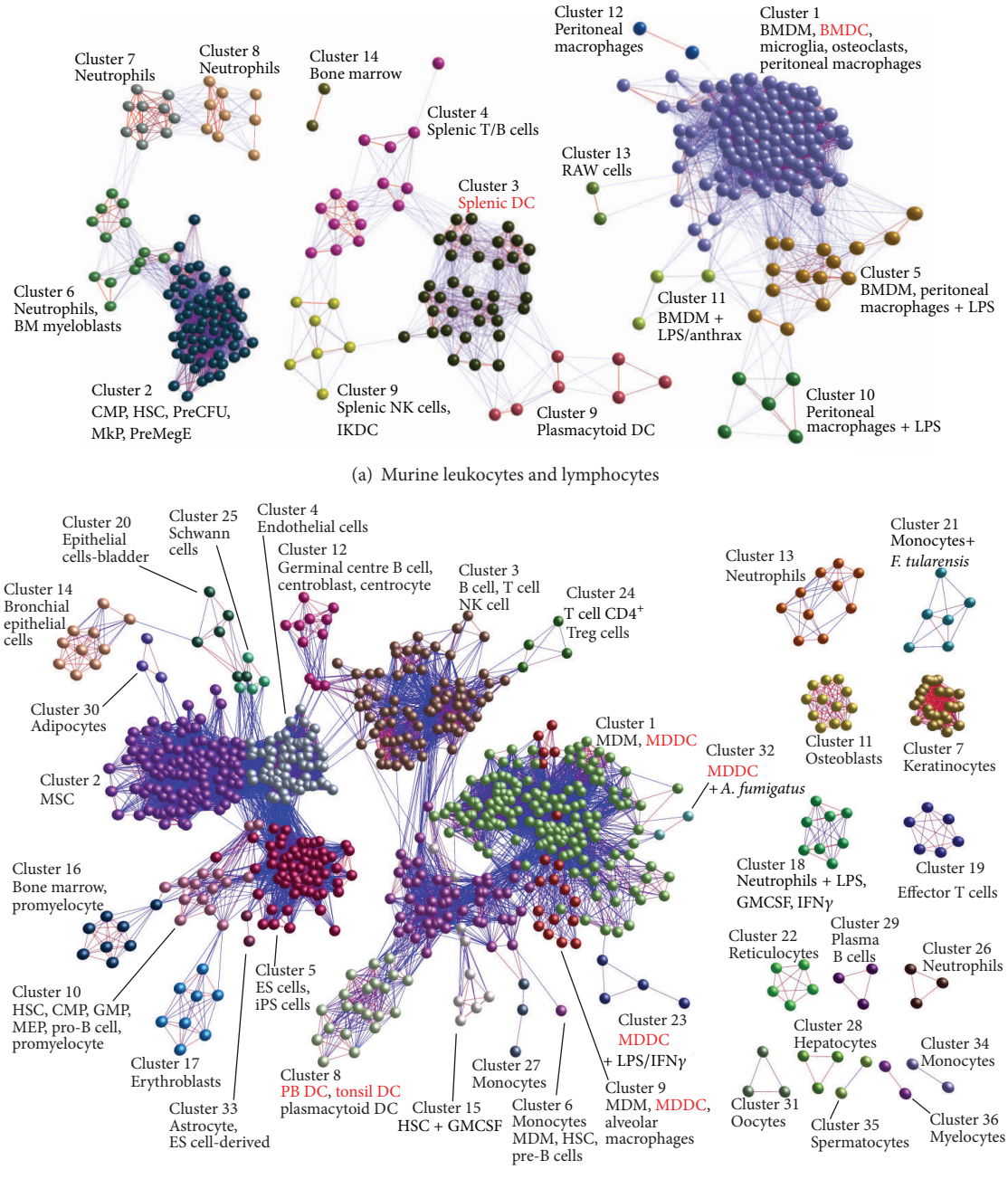


Figure 5: Transcriptional analyses show that mouse bone marrow-derived DC and human monocyte-derived DC are indistinguishable from macrophages. Clustering of cell subsets based upon their global gene expression profiles. In panels "(a)" and "(b)" Pearson correlation matrices were prepared by comparing the microarray data sets derived from all samples used in each study ([11] and [105], resp.). Graphs were then constructed using only those sample-to-sample relationships greater than $r = 0.9$ and clustered with an MCL inflation value of 2.2. Each node represents an individual microarray data set and the edges are coloured on a sliding scale according to the strength of the correlation (red, $r = 1.0$; blue, $r = 0.9$). Each cluster of samples was assigned a different colour. Each of these analyses shows that at the transcriptomic level the *in vitro* bone marrow-derived DC (BMDC) or monocyte-derived DC (MDDC) prepared from mice (panel "(a)") and humans (panel "(b)") are indistinguishable from macrophages and do not cluster with conventional DC enriched from tissues such as the spleen, tonsils, or peripheral blood. The tissue DC, BMDC, and MDDC data sets are highlighted in red font. Reference [11] provides full details about the sources of all the 304 individual microarray data sets used in "(a)." Reference [105] provides full details about the sources of all the 745 microarray data sets used in "(b)." BMDC, bone marrow-derived dendritic cells; BMDM, bone marrow-derived macrophage; anthrax, *Bacillus anthracis* edema toxin; CMP, common myeloid progenitors; ES, embryonic stem cell; GMP, granulocyte monocyte progenitors; HSC, haematopoietic stem cell; IKDC, interferon-producing killer DC; MDDC, monocyte-derived DC; MDM, monocyte-derived macrophage; MEP, megakaryocyte-erythroid progenitor cell; MkP, megakaryocyte progenitors; MSC, mesenchymal stem cells; NK, natural killer; PreCFU-E, preerythroid progenitors; PreMegE, premegakaryocyte/erythroid; PB, peripheral blood; Treg, regulatory T cell. Panel "(a)" is reproduced from [11] with permission from Elsevier. Panel "(b)" is reproduced from [105] under the terms of the Creative Commons Attribution License 2.0.

not observed arguing against the cell-associated haematogenous spread of prions into the CNS. Studies in mice also show that CCR2-deficiency and absence of recruitment of circulating monocytes do not significantly impact on prion disease pathogenesis within the CNS [103]. The depletion of sympathetic nerves dramatically impedes the spread of prions from lymphoid tissues to the CNS [10]. Conversely, prion pathogenesis after peripheral exposure is exacerbated by treatments which increase the density of sympathetic nerves in lymphoid tissues [10] or in mice in which the distance between FDC and sympathetic nerves is reduced [88]. These findings are consistent with the conclusion that prions initially infect the CNS via their spread along peripheral nerves rather than direct haematogenous transfer.

11. Conclusions

11.1. The Many Faces of DC during Prion Disease. As described above, DC have been proposed to exert a diverse range of contrasting effects on prion disease pathogenesis which may have a significant outcome on the spread of infection to the CNS. Some studies have suggested that DC may help to protect the host against infection by attempting to sequester and destroy the prions. Others suggest that prions may exploit the migratory characteristics of prions to facilitate their efficient propagation from the site of exposure to the lymphoid tissues. DC may also play an important role in the subsequent transfer of prions to the CNS by bridging the gap between the immune and peripheral nervous systems (Figure 4).

11.2. DC or Not DC? While it is evident from data described above that the actions of certain MNP populations may significantly influence the outcome of a peripheral prion infection, it is uncertain whether the cells involved actually are DC. Indeed there is much controversy over whether DC and macrophages can be separated based on either their functions or transcriptomes [13, 104]. Most of the studies in experimental mice have defined conventional DC based on their expression of a limited number of cell surface markers such as the integrin CD11c (integrin alpha x (Itgax)). Murine conventional DC do express CD11c highly, but this integrin is not restricted to these cells as most MNP express CD11c, including the majority of the MNP within the intestine [12]. Large numbers of DC-like cells can be prepared *in vitro* following the treatment of bone marrow cells or monocytes with GM-CSF and IL-4. The cells obtained from these preparations do share many typical characteristics of conventional DC, such as expression of high levels of CD11c and potent antigen-presenting activity, but macrophages can also share these characteristics [13, 104]. Furthermore, at the transcriptomic level these *in vitro* bone marrow-derived or monocyte-derived DC prepared from mice and humans are indistinguishable from macrophages and do not cluster with conventional DC enriched from tissues [11, 13, 105] (Figure 5). While data clearly show that MNP may have many important effects during prion infection, further studies are necessary to distinguish between the separate roles of DC and macrophages in disease pathogenesis in experimental rodents and natural host species.

11.3. DC-Based Antiprion Immunotherapy. As well as playing an important role in the establishment of a peripherally acquired prion infection, current data suggest that conventional DC may potentially be manipulated in order to provide immunotherapeutic protection against peripherally acquired prion diseases. As proof-of-principal studies have shown, a conventional DC-based immunotherapy approach can overcome host tolerance towards PrP^C and impede peripheral prion disease pathogenesis [54, 106, 107]. Finally, the early accumulation of prions in the draining lymphoid tissue is impeded and disease susceptibility reduced in the absence of $CD11c^+$ cells at the time of exposure [22–24, 39]. Therefore, the identification of the molecular factors which influence the handling of prions by $CD11c^+$ MNP may reveal novel targets for therapeutic intervention in the initial phase of infection with these invariably fatal neurodegenerative diseases.

Abbreviations

CJD: Creutzfeldt-Jakob disease
CNS: Central nervous system
CR: Complement receptor
DC: Dendritic cells
FDC: Follicular dendritic cells
IFN: Interferon
MLN: Mesenteric lymph nodes
MNP: Mononuclear phagocytes
PrP: Prion protein
TNT: Tunnelling nanotubes.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by project (BB/F019726-1 and BB/G003947/1) and Institute Strategic Programme Grant funding (BB/J004332/1 and BB/J004227/1) from the Biotechnological and Biological Sciences Research Council.

References

[1] G. Legname, I. V. Baskakov, H.-O. B. Nguyen et al., "Synthetic mammalian prions," *Science*, vol. 305, no. 5684, pp. 673–676, 2004.

[2] F. Wang, X. Wang, C.-G. Yuan, and J. Ma, "Generating a prion with bacterially expressed recombinant prion protein," *Science*, vol. 327, no. 5969, pp. 1132–1135, 2010.

[3] D. S. Donaldson, K. J. Else, N. A. Mabbott, and B. Caughey, "The gut-associated lymphoid tissues in the small intestine, not the large intestine, play a major role in oral prion disease pathogenesis," *Journal of Virology*, vol. 89, no. 18, pp. 9532–9547, 2015.

[4] N. A. Mabbott, F. Mackay, F. Minns, and M. E. Bruce, "Temporary inactivation of follicular dendritic cells delays neuroinvasion of scrapie," *Nature Medicine*, vol. 6, no. 7, pp. 719–720, 2000.

[5] F. Montrasio, R. Frigg, M. Glatzel et al., "Impaired prion replication in spleens of mice lacking functional follicular dendritic cells," *Science*, vol. 288, no. 5469, pp. 1257–1259, 2000.

[6] L. McCulloch, K. L. Brown, B. M. Bradford et al., "Follicular dendritic cell-specific prion protein (PrP^C) expression alone is sufficient to sustain prion infection in the spleen," *PLoS Pathogens*, vol. 7, no. 12, Article ID e1002402, 2011.

[7] M. Beekes and P. A. McBride, "Early accumulation of pathological PrP in the enteric nervous system and gut-associated lymphoid tissue of hamsters orally infected with scrapie," *Neuroscience Letters*, vol. 278, no. 3, pp. 181–184, 2000.

[8] P. Kujala, C. R. Raymond, M. Romeijn et al., "Prion uptake in the gut: identification of the first uptake and replication sites," *PLoS Pathogens*, vol. 7, no. 12, Article ID e1002449, 2011.

[9] P. A. McBride, W. J. Schulz-Schaeffer, M. Donaldson et al., "Early spread of scrapie from the gastrointestinal tract to the central nervous system involves autonomic fibers of the splanchnic and vagus nerves," *Journal of Virology*, vol. 75, no. 19, pp. 9320–9327, 2001.

[10] M. Glatzel, F. L. Heppner, K. M. Albers, and A. Aguzzi, "Sympathetic innervation of lymphoreticular organs is rate limiting for prion neuroinvasion," *Neuron*, vol. 31, no. 1, pp. 25–34, 2001.

[11] N. A. Mabbott, J. K. Baillie, D. A. Hume, and T. C. Freeman, "Meta-analysis of lineage-specific gene expression signatures in mouse leukocyte populations," *Immunobiology*, vol. 215, no. 9-10, pp. 724–736, 2010.

[12] B. M. Bradford, D. P. Sester, D. A. Hume, and N. A. Mabbott, "Defining the anatomical localisation of subsets of the murine mononuclear phagocyte system using integrin alpha X (Itgax, CD11c) and colony stimulating factor 1 receptor (Csf1r, CD115) expression fails to discriminate dendritic cells from macrophages," *Immunobiology*, vol. 216, no. 11, pp. 1228–1237, 2011.

[13] D. A. Hume, N. Mabbott, S. Raza, and T. C. Freeman, "Can DCs be distinguished from macrophages by molecular signatures?" *Nature Immunology*, vol. 14, no. 3, pp. 187–189, 2013.

[14] S. A. Houston, V. Cerovic, C. Thomson, J. Brewer, A. M. Mowat, and S. Milling, "The lymph nodes draining the small intestine and colon are anatomically separate and immunologically distinct," *Mucosal Immunology*, 2015.

[15] J. Banchereau, F. Briere, C. Caux et al., "Immunobiology of dendritic cells," *Annual Review of Immunology*, vol. 18, pp. 767–811, 2000.

[16] T. G. Phan, J. A. Green, Y. Xu, and J. G. Cyster, "Immune complex relay by subcapsular sinus macrophages and noncognate B cells drives antibody affinity maturation," *Nature Immunology*, vol. 10, no. 7, pp. 786–793, 2009.

[17] M. Wykes, A. Pombo, C. Jenkins, and G. G. MacPherson, "Dendritic cells interact directly with naive B lymphocytes to transfer antigen and initiate class switching in a primary T-dependent response," *Journal of Immunology*, vol. 161, no. 3, pp. 1313–1319, 1998.

[18] N. A. Mabbott, J. K. Baillie, A. Kobayashi et al., "Expression of mesenchyme-specific gene signatures by follicular dendritic cells: insights from the meta-analysis of microarray data from multiple mouse cell populations," *Immunology*, vol. 133, no. 4, pp. 482–498, 2011.

[19] N. J. Krautler, V. Kana, J. Kranich et al., "Follicular dendritic cells emerge from ubiquitous perivascular precursors," *Cell*, vol. 150, no. 1, pp. 194–206, 2012.

[20] 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.

[21] R. M. Steinman, A. Granelli-Piperno, M. Pope et al., "The interaction of immunodeficiency viruses with dendritic cells," *Current Topics in Microbiology and Immunology*, vol. 276, pp. 1–30, 2003.

[22] C. R. Raymond, P. Aucouturier, and N. A. Mabbott, "*In vivo* depletion of $CD11c^+$ cells impairs scrapie agent neuroinvasion from the intestine," *Journal of Immunology*, vol. 179, no. 11, pp. 7758–7766, 2007.

[23] S. Cordier-Dirikoc and J. Chabry, "Temporary depletion of $CD11c^+$ dendritic cells delays lymphoinvasion after intraperitonal scrapie infection," *Journal of Virology*, vol. 82, no. 17, pp. 8933–8936, 2008.

[24] S. Sethi, K. M. Kerksiek, T. Brocker, and H. Kretzschmar, "Role of the $CD8^+$ dendritic cell subset in transmission of prions," *Journal of Virology*, vol. 81, no. 9, pp. 4877–4880, 2007.

[25] J. Mohan, J. Hopkins, and N. A. Mabbott, "Skin-derived dendritic cells acquire and degrade the scrapie agent following *in vitro* exposure," *Immunology*, vol. 116, no. 1, pp. 122–133, 2005.

[26] J. Mohan, M. E. Bruce, and N. A. Mabbott, "Neuroinvasion by scrapie following inoculation via the skin is independent of migratory Langerhans cells," *Journal of Virology*, vol. 79, no. 3, pp. 1888–1897, 2005.

[27] C. Rybner-Barnier, C. Jacquemot, C. Cuche et al., "Processing of the bovine spongiform encephalopathy-specific prion protein by dendritic cells," *Journal of Virology*, vol. 80, no. 10, pp. 4656–4663, 2006.

[28] K. M. Luhr, R. P. A. Wallin, H.-G. Ljunggren, P. Löw, A. Taraboulos, and K. Kristensson, "Processing and degradation of exogenous prion protein by $CD11c^+$ myeloid dendritic cells in vitro," *Journal of Virology*, vol. 76, no. 23, pp. 12259–12264, 2002.

[29] K. M. Luhr, E. K. Nordström, P. Löw, H.-G. Ljunggren, A. Taraboulos, and K. Kristensson, "Scrapie protein degradation by cysteine proteases in $CD11c^+$ dendritic cells and GT1-1 neuronal cells," *Journal of Virology*, vol. 78, no. 9, pp. 4776–4782, 2004.

[30] R. I. Carp and S. M. Callahan, "Effect of mouse peritoneal macrophages on scrapie infectivity during extended in vitro incubation," *Intervirology*, vol. 17, no. 4, pp. 201–207, 1982.

[31] Y. Sassa, Y. Inoshima, and N. Ishiguro, "Bovine macrophage degradation of scrapie and BSE PrP^{Sc}," *Veterinary Immunology and Immunopathology*, vol. 133, no. 1, pp. 33–39, 2010.

[32] P. Aucouturier, F. Geissmann, D. Damotte et al., "Infected splenic dendritic cells are sufficient for prion transmission to the CNS in mouse scrapie," *Journal of Clinical Investigation*, vol. 108, no. 5, pp. 703–708, 2001.

[33] E. Levavasseur, P. Metharom, G. Dorban et al., "Experimental scrapie in '*plt*' mice: an assessment of the role of dendritic-cell migration in the pathogenesis of prion diseases," *Journal of General Virology*, vol. 88, no. 8, pp. 2353–2360, 2007.

[34] F.-P. Huang, C. F. Farquhar, N. A. Mabbott, M. E. Bruce, and G. G. MacPherson, "Migrating intestinal dendritic cells transport PrP^{Sc} from the gut," *Journal of General Virology*, vol. 83, pp. 267–271, 2002.

[35] C. R. Raymond and N. A. Mabbott, "Assessing the involvement of migratory dendritic cells in the transfer of the scrapie agent

from the immune to peripheral nervous systems," *Journal of Neuroimmunology*, vol. 187, no. 1-2, pp. 114–125, 2007.

[36] R. Castro-Seoane, H. Hummerich, T. Sweeting et al., "Plasmacytoid dendritic cells sequester high prion titres at early stages of prion infection," *PLoS Pathogens*, vol. 8, no. 2, Article ID e1002538, 2012.

[37] V. Beringue, M. Demoy, C. I. Lasmézas et al., "Role of spleen macrophages in the clearance of scrapie agent early in pathogenesis," *Journal of Pathology*, vol. 190, no. 4, pp. 495–502, 2000.

[38] T. Maignien, M. Shakweh, P. Calvo et al., "Role of gut macrophages in mice orally contaminated with scrapie or BSE," *International Journal of Pharmaceutics*, vol. 298, no. 2, pp. 293–304, 2005.

[39] G. J. Wathne, A. Kissenpfennig, B. Malissen, C. Zurzolo, and N. A. Mabbott, "Determining the role of mononuclear phagocytes in prion neuroinvasion from the skin," *Journal of Leukocyte Biology*, vol. 91, no. 5, pp. 817–828, 2012.

[40] L. Delamarre, M. Pack, H. Chang, I. Mellman, and E. S. Trombetta, "Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate," *Science*, vol. 307, no. 5715, pp. 1630–1634, 2005.

[41] A. Bergtold, D. D. Desai, A. Gavhane, and R. Clynes, "Cell surface recycling of internalized antigen permits dendritic cell priming of B cells," *Immunity*, vol. 23, no. 5, pp. 503–514, 2005.

[42] J. Burthem, B. Urban, A. Pain, and D. J. Roberts, "The normal cellular prion protein is strongly expressed by myeloid dendritic cells," *Blood*, vol. 98, no. 13, pp. 3733–3738, 2001.

[43] K. Miyazawa, T. Kanaya, S. Tanaka et al., "Immunohistochemical characterization of cell types expressing the cellular prion protein in the small intestine of cattle and mice," *Histochemistry and Cell Biology*, vol. 127, no. 3, pp. 291–301, 2007.

[44] S. Cordier-Dirikoc, N. Zsürger, J. Cazareth, B. Ménard, and J. Chabry, "Expression profiles of prion and doppel proteins and of their receptors in mouse splenocytes," *European Journal of Immunology*, vol. 38, no. 8, pp. 2131–2141, 2008.

[45] K. L. Brown, K. Stewart, D. L. Ritchie et al., "Scrapie replication in lymphoid tissues depends on prion protein-expressing follicular dendritic cells," *Nature Medicine*, vol. 5, no. 11, pp. 1308–1312, 1999.

[46] M. A. Klein, R. Frigg, A. J. Raeber et al., "PrP expression in B lymphocytes is not required for prion neuroinvasion," *Nature Medicine*, vol. 4, no. 12, pp. 1429–1433, 1998.

[47] J. Mohan, K. L. Brown, C. F. Farquhar, M. E. Bruce, and N. A. Mabbott, "Scrapie transmission following exposure through the skin is dependent on follicular dendritic cells in lymphoid tissues," *Journal of Dermatological Science*, vol. 35, no. 2, pp. 101–111, 2004.

[48] C. Loeuillet, C. Lemaire-Vieille, P. Naquet, M.-F. Cesbron-Delauw, J. Gagnon, and J.-Y. Cesbron, "Prion replication in the hematopoietic compartment is not required for neuroinvasion in scrapie mouse model," *PLoS ONE*, vol. 5, no. 10, Article ID e13166, 2010.

[49] L. McCulloch, K. L. Brown, and N. A. Mabbott, "Ablation of the cellular prion protein, PrP^C, specifically on follicular dendritic cells has no effect on their maturation or function," *Immunology*, vol. 138, no. 3, pp. 246–257, 2013.

[50] M. Nuvolone, V. Kana, G. Hutter et al., "SIRPα polymorphisms, but not the prion protein, control phagocytosis of apoptotic cells," *The Journal of Experimental Medicine*, vol. 210, no. 12, pp. 2539–2552, 2013.

[51] C. Ballerini, P. Gourdain, V. Bachy et al., "Functional implication of cellular prion protein in antigen-driven interactions between T cells and dendritic cells," *The Journal of Immunology*, vol. 176, no. 12, pp. 7254–7262, 2006.

[52] D. D. Richardson, S. Tol, E. Valle-Encinas et al., "The prion protein inhibits monocytic cell migration by stimulating β1 integrin adhesion and uropod formation," *Journal of Cell Science*, vol. 128, no. 16, pp. 3018–3029, 2015.

[53] N. A. Mabbott, "Prospects for safe and effective vaccines against prion diseases," *Expert Review of Vaccines*, vol. 14, no. 1, pp. 1–4, 2014.

[54] V. Bachy, C. Ballerini, P. Gourdain et al., "Mouse vaccination with dendritic cells loaded with prion protein peptides overcomes tolerance and delays scrapie," *Journal of General Virology*, vol. 91, no. 3, pp. 809–820, 2010.

[55] S.-J. L. Wu, G. Grouard-Vogel, W. Sun et al., "Human skin Langerhans cells are targets of dengue virus infection," *Nature Medicine*, vol. 6, no. 7, pp. 816–820, 2000.

[56] L.-J. Ho, J.-J. Wang, M.-F. Shaio et al., "Infection of human dendritic cells by dengue virus causes cell maturation and cytokine production," *The Journal of Immunology*, vol. 166, no. 3, pp. 1499–1506, 2001.

[57] A. W. S. Ho, N. Prabhu, R. J. Betts et al., "Lung $CD103^+$ dendritic cells efficiently transport influenza virus to the lymph node and load viral antigen onto MHC class I for presentation to CD8 T cells," *Journal of Immunology*, vol. 187, no. 11, pp. 6011–6021, 2011.

[58] H. Saeki, A. M. Moore, M. J. Brown, and S. T. Hwang, "Cutting edge: secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes," *Journal of Immunology*, vol. 162, no. 5, pp. 2472–2475, 1999.

[59] M. H. Jang, N. Sougawa, T. Tanaka et al., "CCR7 is critically important for migration of dendritic cells in intestinal lamina propria to mesenteric lymph nodes," *The Journal of Immunology*, vol. 176, no. 2, pp. 803–810, 2006.

[60] B. R. Glaysher and N. A. Mabbott, "Role of the GALT in scrapie agent neuroinvasion from the intestine," *Journal of Immunology*, vol. 178, no. 6, pp. 3757–3766, 2007.

[61] M. A. Klein, R. Frigg, E. Flechsig et al., "A crucial role for B cells in neuroinvasive scrapie," *Nature*, vol. 390, no. 6661, pp. 687–690, 1997.

[62] M. Jeffrey, L. González, A. Espenes et al., "Transportation of prion protein across the intestinal mucosa of scrapie-susceptible and scrapie-resistant sheep," *Journal of Pathology*, vol. 209, no. 1, pp. 4–14, 2006.

[63] S. J. Ryder, G. E. Dexter, L. Heasman, R. Warner, and S. J. Moore, "Accumulation and dissemination of prion protein in experimental sheep scrapie in the natural host," *BMC Veterinary Research*, vol. 5, article 9, 2009.

[64] C. P. Åkesson, C. M. Press, M. A. Tranulis et al., "Phenotypic characterization of cells participating in transport of prion protein aggregates across the intestinal mucosa of sheep," *Prion*, vol. 6, no. 3, pp. 261–275, 2012.

[65] L. Martínez-Pomares and S. Gordon, "Potential role of the mannose receptor in antigen transport," *Immunology Letters*, vol. 65, no. 1-2, pp. 9–13, 1999.

[66] C. Berney, S. Herren, C. A. Power, S. Gordon, L. Martinez-Pomares, and M. H. Kosco-Vilbois, "A member of the dendritic cell family that enters B cell follicles and stimulates primary antibody responses identified by a mannose receptor fusion

protein," *Journal of Experimental Medicine*, vol. 190, no. 6, pp. 851–860, 1999.

[67] M. D. Gunn, V. N. Ngo, K. M. Ansel, E. H. Ekland, J. G. Cyster, and L. T. Williams, "A B-cell-homing chemokine made in lymphoid follicles activates Burkitt's lymphoma receptor-1," *Nature*, vol. 391, no. 6669, pp. 799–803, 1998.

[68] K. M. Ansel, V. N. Ngo, P. L. Hyman et al., "A chemokine-driven positive feedback loop organizes lymphoid follicles," *Nature*, vol. 406, no. 6793, pp. 309–314, 2000.

[69] P. Yu, Y. Wang, R. K. Chin et al., "B cells control the migration of a subset of dendritic cells into B cell follicles via CXC chemokine ligand 13 in a lymphotoxin-dependent fashion," *Journal of Immunology*, vol. 168, no. 10, pp. 5117–5123, 2002.

[70] H. Saeki, M.-T. Wu, E. Olasz, and S. T. Hwang, "A migratory population of skin-derived dendritic cells expresses CXCR5, responds to B lymphocyte chemoattractant in vitro, and co-localizes to B cell zones in lymph nodes in vivo," *European Journal of Immunology*, vol. 30, no. 10, pp. 2808–2814, 2000.

[71] S. F. Gonzalez, V. Lukacs-Kornek, M. P. Kuligowski et al., "Capture of influenza by medullary dendritic cells via SIGN-R1 is essential for humoral immunity in draining lymph nodes," *Nature Immunology*, vol. 11, no. 5, pp. 427–434, 2010.

[72] B. Michel, C. Meyerett-Reid, T. Johnson et al., "Incunabular immunological events in prion trafficking," *Scientific Reports*, vol. 2, article 440, 2012.

[73] A. Gossner, N. Hunter, and J. Hopkins, "Role of lymph-borne cells in the early stages of scrapie agent dissemination from the skin," *Veterinary Immunology and Immunopathology*, vol. 109, no. 3-4, pp. 267–278, 2006.

[74] N. C. Kaneider, A. Kaser, S. Dunzendorfer, H. Tilg, and C. J. Wiedermann, "Sphingosine kinase-dependent migration of immature dendritic cells in response to neurotoxic prion protein fragment," *Journal of Virology*, vol. 77, no. 9, pp. 5535–5539, 2003.

[75] N. A. Mabbott, M. E. Bruce, M. Botto, M. J. Walport, and M. B. Pepys, "Temporary depletion of complement component C3 or genetic deficiency of C1q significantly delays onset of scrapie," *Nature Medicine*, vol. 7, no. 4, pp. 485–487, 2001.

[76] M. A. Klein, P. S. Kaeser, P. Schwarz et al., "Complement facilitates early prion pathogenesis," *Nature Medicine*, vol. 7, no. 4, pp. 488–492, 2001.

[77] A. Flores-Langarica, Y. Sebti, D. A. Mitchell, R. B. Sim, and G. G. MacPherson, "Scrapie pathogenesis: the role of complement C1q in scrapie agent uptake by conventional dendritic cells," *Journal of Immunology*, vol. 182, no. 3, pp. 1305–1313, 2009.

[78] S. R. B. Sunkesula, X. Luo, D. Das, A. Singh, and N. Singh, "Iron content of ferritin modulates its uptake by intestinal epithelium: implications for co-transport of prions," *Molecular Brain*, vol. 3, article 14, 2010.

[79] B. Reizis, A. Bunin, H. S. Ghosh, K. L. Lewis, and V. Sisirak, "Plasmacytoid dendritic cells: recent progress and open questions," *Annual Review of Immunology*, vol. 29, pp. 163–183, 2011.

[80] U. Yrlid, V. Cerovic, S. Milling et al., "Plasmacytoid dendritic cells do not migrate in intestinal or hepatic lymph," *Journal of Immunology*, vol. 177, no. 9, pp. 6115–6121, 2006.

[81] G. Martínez del Hoyo, M. López-Bravo, P. Metharom, C. Ardavín, and P. Aucouturier, "Prion protein expression by mouse dendritic cells is restricted to the nonplasmacytoid subsets and correlates with the maturation state," *Journal of Immunology*, vol. 177, no. 9, pp. 6137–6142, 2006.

[82] M. Katz and H. Koprowski, "Failure to demonstrate a relationship between scrapie and production of interferon in mice," *Nature*, vol. 219, no. 5154, pp. 639–640, 1968.

[83] R. Field, S. Campion, C. Warren, C. Murray, and C. Cunningham, "Systemic challenge with the TLR3 agonist poly I:C induces amplified IFNα/β and IL-1β responses in the diseased brain and exacerbates chronic neurodegeneration," *Brain, Behavior, and Immunity*, vol. 24, no. 6, pp. 996–1007, 2010.

[84] C. A. Baker, Z. Y. Lu, and L. Manuelidis, "Early induction of interferon-responsive mRNAs in Creutzfeldt-Jakob disease," *Journal of NeuroVirology*, vol. 10, no. 1, pp. 29–40, 2004.

[85] M. Worthington, "Interferon system in mice infected with the scrapie agent," *Infection and Immunity*, vol. 6, no. 4, pp. 643–645, 1972.

[86] G. Doré, C. Leclerc, and F. Lazarini, "Treatment by CpG or Flt3-ligand does not affect mouse susceptibility to BSE prions," *Journal of Neuroimmunology*, vol. 197, no. 1, pp. 74–80, 2008.

[87] P.-C. Klöhn, R. Castro-Seoane, and J. Collinge, "Exosome release from infected dendritic cells: a clue for a fast spread of prions in the periphery?" *Journal of Infection*, vol. 67, no. 5, pp. 359–368, 2013.

[88] M. Prinz, M. Helkenwalder, T. Junt et al., "Positioning of follicular dendritic cells within the spleen controls prion neuroinvasion," *Nature*, vol. 425, no. 6961, pp. 957–962, 2003.

[89] A. Flores-Langarica, S. Meza-Perez, J. Calderon-Amador et al., "Network of dendritic cells within the muscular layer of the mouse intestine," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 52, pp. 19039–19044, 2005.

[90] P. A. Muller, B. Koscso, G. M. Rajani et al., "Crosstalk between muscularis macrophages and enteric neurones regulates gastrointestinal motility," *Cell*, vol. 158, no. 2, pp. 300–313, 2014.

[91] K. Gousset, E. Schiff, C. Langevin et al., "Prions hijack tunnelling nanotubes for intercellular spread," *Nature Cell Biology*, vol. 11, no. 3, pp. 328–336, 2009.

[92] C. Langevin, K. Gousset, M. Costanzo, O. Richard-Le Goff, and C. Zurzolo, "Characterization of the role of dendritic cells in prion transfer to primary neurons," *Biochemical Journal*, vol. 431, no. 2, pp. 189–198, 2010.

[93] Y. Tanaka, T. Sadaike, Y. Inoshima, and N. Ishiguro, "Characterization of PrP^{Sc} transmission from immune cells to neuronal cells," *Cellular Immunology*, vol. 279, no. 2, pp. 145–150, 2012.

[94] S. Zhu, G. S. Victoria, L. Marzo, R. Ghosh, and C. Zurzolo, "Prion aggregates transfer through tunneling nanotubes in endocytic vesicles," *Prion*, vol. 9, no. 2, pp. 125–135, 2015.

[95] W. Xu, P. A. Santini, J. S. Sullivan et al., "HIV-1 evades virus-specific IgG2 and IgA responses by targeting systemic and intestinal B cells via long-range intercellular conduits," *Nature Immunology*, vol. 10, no. 9, pp. 1008–1017, 2009.

[96] B. Fevrier, D. Vilette, F. Archer et al., "Cells release prions in association with exosomes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 26, pp. 9683–9688, 2004.

[97] K. Bulloch, M. M. Miller, J. Gal-Toth et al., "CD11c/EYFP transgene illuminates a discrete network of dendritic cells within the embryonic, neonatal, adult, and injured mouse brain," *Journal of Comparative Neurology*, vol. 508, no. 5, pp. 687–710, 2008.

[98] O. Koperek, G. G. Kovács, D. Ritchie, J. W. Ironside, H. Budka, and G. Wick, "Disease-associated prion protein in vessel walls," *The American Journal of Pathology*, vol. 161, no. 6, pp. 1979–1984, 2002.

[99] B. Rosicarelli, B. Serafini, M. Sbriccoli et al., "Migration of dendritic cells into the brain in a mouse model of prion disease," *Journal of Neuroimmunology*, vol. 165, no. 1-2, pp. 114–120, 2005.

[100] A. F. Cintron, N. V. Dalal, J. Dooyema, R. Betarbet, and L. C. Walker, "Transport of cargo from periphery to brain by circulating monocytes," *Brain Research*, vol. 1622, pp. 328–338, 2015.

[101] R. Hasebe, G. J. Raymond, M. Horiuchi, and B. Caughey, "Reaction of complement factors varies with prion strains in vitro and in vivo," *Virology*, vol. 423, no. 2, pp. 205–213, 2012.

[102] S. Sisó, L. González, M. Jeffrey, S. Martin, F. Chianini, and P. Steele, "Prion protein in kidneys of scrapie-infected sheep," *Veterinary Record*, vol. 159, no. 10, pp. 327–328, 2006.

[103] D. Gómez-Nicola, S. T. T. Schetters, and V. Hugh Perry, "Differential role of CCR2 in the dynamics of microglia and perivascular macrophages during prion disease," *Glia*, vol. 62, no. 7, pp. 1041–1052, 2014.

[104] D. A. Hume, "Macrophages as APC and the dendritic cell myth," *The Journal of Immunology*, vol. 181, no. 9, pp. 5829–5835, 2008.

[105] N. A. Mabbott, J. K. Baillie, H. Brown, T. C. Freeman, and D. A. Hume, "An expression atlas of human primary cells: inference of gene function from coexpression networks," *BMC Genomics*, vol. 14, no. 1, article 632, 2013.

[106] M. B. Rosset, A. Sacquin, S. Lecollinet et al., "Dendritic cell-mediated-immunization with xenogenic PrP and adenoviral vectors breaks tolerance and prolongs mice survival against experimental scrapie," *PLoS ONE*, vol. 4, no. 3, Article ID e4917, 2009.

[107] C. Carnaud and V. Bachy, "Cell-based immunotherapy of prion diseases by adoptive transfer of antigen-loaded dendritic cells or antigen-primed CD4+ T lymphocytes," *Prion*, vol. 4, no. 2, pp. 66–71, 2010.