Review Article

Molecular and Cellular Pathways of Immunoglobulin G Activity In Vivo

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In retrospect, the therapeutic potential of immunoglobulins was first demonstrated by von Behring and Kitasato in the late nineteenth century by protecting mice from the lethal effects caused by tetanus and diphtheria toxin via injection of a hyperimmune serum generated in rabbits. Even today, hyperimmune sera generated from human donors with high serum titers against a certain pathogen are still in use as a means of providing passive protection. More importantly, therapeutic antibodies specific for malignant or autoreactive cells have become included in the standard of care in diseases such as breast cancer and malignant lymphoma. Despite this clinical success, we are only at the beginning of understanding the precise molecular and cellular pathways responsible for immunoglobulin G (IgG) activity in vivo. Since then, an enormous amount of information about the mechanism of IgG activity has been obtained in various model systems. The aim of this review is to provide a comprehensive overview of our current understanding of how IgG antibodies mediate their activity in vivo and how we can use this knowledge to enhance the activity of therapeutic antibodies or block the proinflammatory and tissue pathology inducing activity of autoantibodies.

1. Introduction

Antibodies of the IgG isotype are an essential component of our armament against pathogenic microorganisms. IgG binding to invading microorganisms can block their binding to cellular receptors providing the basis for the so-called “sterile” immunity, which protects us from reinfection with highly cytopathic viruses such as influenza. On the other hand, IgG binding to bacteria makes them more visible for phagocytic cells, allowing removing them or their toxic products from the body. Apart from this protective function, autoantibodies directed against self-antigens are a major cause of tissue inflammation or removal of platelets and red blood cells resulting in autoimmune diseases such as systemic lupus erythematosus (SLE), chronic inflammatory demyelinating polyneuropathy (CIDP), skin blistering diseases, immunothrombocytopenia (ITP), and autoimmune hemolytic anemia (AIHA) [1–3]. Finally the potent cytotoxic function of IgG molecules having the capacity to find and destroy virtually any cell within the body is used therapeutically in the form of tumor-specific cytotoxic antibodies,

exemplified by CD20, Her2/Neu, or CD52 specific antibodies [4, 5].

A detailed understanding of the molecular and cellular mechanisms underlying IgG activity are therefore of great importance as this may allow (1) improving therapeutic antibodies and (2) preventing the proinflammatory activity of autoantibodies ultimately allowing the development of novel therapeutic avenues to treat autoimmune disease. Apart from this proinflammatory, cytotoxic, and phagocytic activity of IgG molecules, it has become clear over the recent years that IgG molecules in the form of polyclonal serum IgG preparations pooled from thousands of donors (so called intravenous IgG or IVIg therapy) can also have an active anti-inflammatory activity, which is used in the clinic to treat autoantibody-mediated autoimmune diseases such as ITP, CIDP, Guillain-Barré syndrome, and more recently also skin blistering diseases [6, 7]. Despite not being the major topic of this review, to understand how this paradoxical anti-inflammatory works, it is also critical to understand which molecular and cellular pathways are responsible for the proinflammatory activity of IgG.

2. Effector Pathways Triggered by the IgG Molecule

One of the major findings during the last 20 years was that the IgG Fc-fragment is essential for the majority of the protective and pathogenic activities of immunoglobulin G. In contrast to IgM or IgE, IgG is a family of molecules comprised of four members in mice (IgG1, IgG2a, IgG2b, and IgG3) and humans (IgG1, IgG2, IgG3, and IgG4). One major function of the antibody Fc-fragment is to activate humoral (via the complement system) and cellular responses (via the family of Fc-receptors expressed on innate immune effector cells) of the innate immune system. Thus, IgG molecules are a molecular link connecting the antigen specificity generated by the adaptive immune system to the effector functions provided by the innate immune system, which will be explained in more detail in the next paragraph.

The complement system is comprised of a family of molecules (called C1-C9) which are present in the serum at high levels in an inactive form as so called proenzymes [8]. The most potent immunoglobulin isotypes able to activate the complement system are select IgG subclasses (such as IgG1 and IgG3 in humans and IgG2a, IgG2b, and IgG3 in the mouse) and IgM. To gain this capacity, immunoglobulins have to be bound to their target structure (bacterial cells for example), which will trigger a conformational change and the binding to the first complement component C1q. This will result in the antibody independent activation of all downstream complement components which initiates three major effector pathways. First, certain activated complement components such as C3a and C5a act as potent proinflammatory mediators attracting innate immune effector cells (most forward neutrophils) which express receptors for these anaphylatoxins to the site of infection. Second other complement components such as C3b, for example, are deposited on the surface of bacteria, which results in their opsonisation and recognition via phagocytic cells again expressing cell surface receptors for C3b. Third, the late complement components C6-C9 can polymerize in the membrane of bacteria and hence are referred to as a membrane attack complex (MAC); this can directly lead to bacterial cell death. Apart from this so called classical pathway of complement activation via C1q through IgG and IgM molecules, there is data suggesting that certain IgG variants which we will discuss later, may also be able to activate a nonclassical pathway of complement activation [9–11]. This pathway is normally initiated by the lectin MBL (mannan binding lectin) which can directly recognize mannose rich structures on bacterial surfaces and thus represents an entirely innate effector pathway for the control of bacterial infection. Surprisingly, certain IgG variants overrepresented during chronic inflammatory disorders such as rheumatoid arthritis were also shown to be able to activate this alternative pathway via binding to MBL.

Apart from the complement system, the family of Fcγ-receptors is widely expressed on cells of the innate immune system and on select cell types of the adaptive immune system such as B cells and some subpopulations of T cells [1, 3, 12, 13]. In mice and humans this receptor family consists of several members of which the majority is triggering cell activation

either autonomously (human FcγRIIA/FcγRIIC) or via the interaction with the common Fcγ-chain (mouse FcγRI, III, IV; human FcγRIA, FcγRIIIA), which contains immunoreceptor tyrosine based activatory motifs (ITAM) (Figure 1(a)). Upon ITAM phosphorylation and the recruitment of Syk, downstream signaling pathways including the Ras, BTK, and PLCγ dependent pathways are triggered resulting in cell activation and initiation of effector responses including antibody dependent cellular cytotoxicity, the release of proinflammatory cytokines, and oxygen radicals [1, 3, 12, 13]. Besides these activating FcγRs there is also one inhibitory FcγR called FcγRIIB which has an intracellular immunoreceptor tyrosine based inhibitory motif (ITIM) and which is expressed very broadly [12, 14–16]. Thus, almost all innate immune effector cells express both activating and inhibitory FcγRs, which results in a simultaneous triggering of activating and inhibitory signaling pathways, thereby setting a threshold for cell activation. On the molecular level, FcγRIIB triggering results in the phosphorylation of the ITIM motif thereby creating a docking site for the phosphatase SHIP, which hydrolyses PIP3 and blocks the recruitment of PLCγ to the membrane. The further binding of DOK and SHC results in the inhibition of RAS dependent signaling pathways (Figure 1(b)) [12]. Taking this into account, the actual affinity of IgG to the respective activating and the inhibitory FcγR may be of major importance in determining if more activating or inhibitory signaling pathways are triggered. Cytokines such as IL4, IFNγ, or TNFα can modulate FcγR expression with TH2-cytokines (IL4) resulting in a downmodulation of activating and upregulation of the inhibitory FcγRIIB on innate immune effector cells [17]. TH1 cytokines (IFNγ, TNFα) have the opposite effect and lead to a strong upregulation of activating receptors. Of note, the ligand for the majority of FcγRs is not monomeric IgG but IgG in the form of an immune complex and the size of this complex will determine the strength of the resulting effector function [18]. The only FcγR able to bind to IgG as a single molecule is the high affinity FcγRI, which is therefore usually saturated with IgG on cells expressing this receptor in the blood.

Depending on the respective innate cell type, the set of FcγRs expressed may vary widely. Thus, NK cells in mice and humans express only one activating FcγR (FcγRIIIA in humans and FcγRIII in mice), whereas basophils, eosinophils, and mast cells express the activating FcγRIII and the inhibitory FcγRIIB [13]. The cell types expressing the broadest set of FcγRs are monocytes and tissue resident macrophages on which all three activating and the inhibitory FcγR may be coexpressed in mice and humans. With respect to neutrophils, an interesting difference exists between mice and humans, as human neutrophils express a GPI-anchored form of FcγRIII (called FcγRIIB), which does not have a signaling or cell activating function and does not exist in mice [19]. In contrast, murine neutrophils express two very potent activating Fc-receptors (FcγRIII and FcγRIV) in combination with FcγRIIB. Besides this example, there are other differences in FcγR expression and ligand binding which suggest that care should be taken when trying to directly transfer data obtained in murine model systems

FIGURE 1: Family of FcγRs and FcγR signaling pathways. (a) Shown is the family of mouse and human Fcγ-receptors, which can be distinguished with respect to their capacity to interact either with monomeric IgG or with multimeric immune complexes. Apart from the family of classical Fcγ-receptors, members of the C-type lectin family have been shown to bind to select IgG glycoforms. See text for further details. (b) Depicted are the signaling pathways triggered upon binding of immune complexes to FcγRIII on innate immune effector cells. Upon FcγRIII crosslinking, the ITAM motif in the common Fcγ-chain becomes phosphorylated, thereby creating docking sites for Syk which is responsible for triggering downstream signaling events. See text for further details.

to humans [20]. The same applies for the four different IgG subclasses in mice and humans, which unfortunately have similar names but very frequently an entirely different activity. As we will see later, mouse IgG1, for example, is an IgG subclass with a low activity, whereas human IgG1 has a very pronounced proinflammatory and cytotoxic activity.

Nonetheless, mice have been essential for our basic understanding of how autoantibodies and therapeutic antibodies mediate their activity *in vivo*, which will be discussed in the next paragraphs. With respect to cells of the adaptive immune system, virtually all B cell stages starting from pro-B cells in the bone marrow to plasma cells and memory B cells express the inhibitory Fc γ RIIB [15, 21]. Instead of regulating activating Fc γ Rs, which are not expressed on B cells, Fc γ RIIB modulates signals transmitted via the B cell receptor. Underlining the importance of this activation threshold set by this receptor for B cell activation, Fc γ RIIB deficient mice start to develop autoantibodies and develop a SLE-like autoimmune disease on certain mouse genetic backgrounds [22–26]. In a similar manner nonfunctional Fc γ RIIB alleles or promoter polymorphisms causing a lower expression level have been associated with the development or severity of human and mouse autoimmune diseases and humanized mice transplanted with a human immune system carrying the nonfunctional Fc γ RIIB allele in a homozygous fashion start to develop autoantibodies [13, 26–30]. As this interesting gatekeeper function of Fc γ RIIB is not the main focus of this paper, the interested reader is directed to several excellent recent reviews covering this in greater detail [6, 15, 21].

3. How Autoantibodies Mediate Their Activity Lessons from ITP, AIHA, and Rheumatoid Arthritis

Immunothrombocytopenia (ITP) and autoimmune hemolytic anemia (AIHA) are autoimmune diseases in which IgG antibodies directed against platelets or red blood cells lead to a removal of these blood components from the circulation resulting in a heightened risk of bleeding in the case of ITP or an anemia in the case of AIHA. The cellular process by which platelet and red blood cell removal is achieved is most likely a phagocytosis by cells of the so called mononuclear phagocyte system (MPS), which comprises monocytes, granulocytes, and tissue resident macrophages. This phagocytic process may happen either under noninflammatory conditions without a major release of proinflammatory cytokines and tissue inflammation and thus is a classical type II hypersensitivity reaction or under inflammatory conditions as described for rat-derived antibody clones specific for the platelet fibrinogen receptor gpIIb/IIIa, which cause early signs of lung injury [31–33]. One possible explanation for this difference in concomitant inflammation may be the loss of antigen-antibody complexes from the platelet surface resulting in a free immune complex which deposits in the lung and triggers inflammation. In contrast, in rheumatoid arthritis immune complexes always cause massive organ inflammation and proinflammatory cytokine release (characteristic for a type III

hypersensitivity reaction). The next paragraphs will summarize our current knowledge about the molecular and cellular pathways underlying these two entirely different autoimmune diseases and hypersensitivity reactions.

3.1. Immunothrombocytopenia. In principle several independent pathways may lead to autoantibody dependent immunothrombocytopenia (ITP). These may include platelet clearance via Fc-receptor dependent phagocytosis, via complement receptor mediated phagocytosis upon deposition of activated complement components (such as C3B) on the platelet surface, or via antibody Fc-fragment independent effects such as the activation of platelets. Data from passive mouse models using a panel of rat derived platelet specific antibodies generated by repeated immunization with mouse platelets demonstrated convincingly that the target antigen on the platelet surface plays an important role with respect to the requirement of the antibody Fc-fragment in the process of platelet clearance. For example, autoantibodies directed against GPIIb α depleted platelets in the form of an intact IgG molecule and as a F(ab)2-fragment [34]. In contrast, the activity of a variety of GPIIb/IIIa specific autoantibodies was fully dependent on the IgG Fc-fragment. Yet another set of platelet specific IgG autoantibodies directed against GPV did not induce any platelet depletion regardless of the IgG subclass and despite being bound to the platelet surface. While this set of data elucidates the impact of the target antigen on the platelet with respect to the likelihood of inducing an acute ITP, it may not reflect the activity of autoantibodies developing naturally in affected individuals in mice and humans. Indeed, a clinical trial performed with a therapeutic monoclonal antibody blocking the interaction of immune complexes with human Fc γ RIIIA (CD16) was very efficient at restoring platelet counts in ITP patients and a similar effect was observed in mice transgenic for the respective human Fc γ R [35, 36]. With respect to mouse model systems, the use of naturally developing platelet specific antibodies and the generation of mouse strains deficient in components of the Fc γ R and complement pathway provided some fascinating insights into the molecular and cellular requirements for platelet depletion via autoantibodies of the IgG isotype [8, 37–39]. The crucial role of the family of Fc γ Rs in this process was first demonstrated by Clynes and colleagues using a naturally occurring platelet specific antibody (clone 6A6) derived from NZWxBXSB F1 offspring mice [40, 41]. By using a mouse strain deficient for all activating Fc γ Rs, the so called common Fc γ -chain deficient mouse, the authors could show that this platelet specific mouse IgG2a antibody was no longer able to induce platelet removal. These results were confirmed later by other studies and similar results were obtained with rat derived platelet specific antibodies such as the rat IgG1 clone MWreg30 [10, 31, 42–44]. Quite unexpectedly, mice deficient in the complement components C3, C4, or the complement receptor 2 were not protected from platelet removal suggesting that at least under these experimental conditions complement dependent phagocytosis of autoantibody opsonized platelets was not a major factor contributing to platelet depletion [44]. Besides this

generalized requirement for Fc γ Rs, a very interesting IgG subclass specific dependence on select members of the family of activating Fc γ Rs was noted. Thus, mouse IgG1 platelet specific antibodies as well as their rat IgG1 counterparts were fully dependent on the presence of mouse Fc γ RIII. In contrast, the IgG2a subclass showed a codependence on the activating Fc γ Rs I and IV, whereas IgG2b platelet specific antibodies were mediating their activity largely through Fc γ RIV [33, 44, 45]. Moreover, an IgG subclass specific activity was noted when using IgG switch variants of the 6A6 platelet specific antibody. When injected at the same dose, the IgG1 subclass was able to deplete about 50% of the platelets whereas IgG2a and IgG2b were much more active and resulted in a removal of about 80–90% of the platelets. IgG3, in contrast, was not able to cause any significant platelet depletion [44]. As we will see later, similar observations were made in many different antibody dependent model systems in vivo [17, 44, 46, 47]. This differential activity correlated well with the actual affinities of the different IgG subclasses to the respective activating Fc γ Rs. In addition, IgG1 had a higher affinity for the inhibitory Fc γ RIIB than for the activating Fc γ RIII, which may be an additional reason for its lower activity. Indeed, IgG1 had a much higher platelet depleting activity in Fc γ RIIB deficient mice, suggesting that IgG1 activity is tightly regulated by the threshold set through the inhibitory Fc-receptor [44]. In a similar manner, the activity of IgG2a and IgG2b subclasses was enhanced in the absence of Fc γ RIIB, albeit at a lower level. As the affinity of the different IgG subclasses for their respective activating and the inhibitory Fc γ RIIB correlated very well with the observed activity in vivo, we introduced the concept of the so called A/I ratio in 2005 allowing predicting the activity of a given IgG subclass in vivo based on its affinity for Fc γ Rs in vitro [17]. In detail, this ratio is calculated by dividing the affinity of an IgG subclass for the activating Fc γ R with the affinity for the inhibitory Fc γ RIIB. Fully consistent with this concept, IgG glycovariants with enhanced binding to the activating but unchanged affinity for the inhibitory Fc γ R translated into a higher cytotoxic activity in vivo [44].

The importance of Fc γ RIIB in modulating the activity of platelet specific autoantibodies may also be underlined by the fact that a clinically very efficient treatment for ITP is critically dependent on the presence of this receptor [36, 48–53]. Quite interestingly, this treatment is nothing else but the infusion of very high doses of pooled serum IgG preparations from several ten thousand donors (intravenous immunoglobulin G or IVIg therapy), which is used to suppress a variety of autoimmune diseases besides ITP. Although we cannot provide an in depth overview of the detailed molecular and cellular pathway underlying this active anti-inflammatory activity of IgG in this review, there is convincing evidence that the IgG Fc-fragment and especially certain IgG glycosylation variants containing sialic acid residues are essential for this immunosuppressive activity [6, 48, 54, 55]. Upon injection of IVIg, an upregulation of Fc γ RIIB and in some studies even a downmodulation of activating Fc γ Rs could be observed which will result in a higher threshold for innate immune effector cell activation. In line with this concept, animals deficient in Fc γ RIIB were no longer protected by IVIG from

autoantibody induced platelet depletion [36, 49, 52, 53]. For more details about this anti-inflammatory and immunomodulatory activity of IgG, the interested reader is directed to several excellent reviews covering this topic in greater detail [6, 7, 56–58]. Further supporting a crucial role of Fc γ Rs in ITP, a recent clinical trial with small molecule inhibitors of the signaling pathways triggered by activating Fc γ Rs was quite successful in preventing platelet depletion in mice and humans [59].

Instead we will focus our attention on the effector cells responsible for IgG mediated platelet depletion. Given the critical role of Fc γ Rs for platelet removal, an involvement of cells of the mononuclear phagocytic system seemed very plausible. Indeed, resident macrophages of the spleen and liver have been suggested to be the major cell types responsible for phagocytosis of opsonized platelets. Moreover, the removal of the spleen is a viable therapeutic option to ameliorate chronic ITP and raise platelet counts to a level which prevents acute bleeding events and injection of radiolabeled platelets has identified the spleen and liver as potential sites for platelet clearance [60–64]. However, roughly 30% of patients are refractory to splenectomy and initially responding patients may finally relapse with ITP suggesting that cell types not residing in the spleen may be responsible for platelet removal [60]. As we have discussed before, data from previous studies suggested that especially Fc γ RIV and Fc γ RI are critical for platelet phagocytosis in mice, allowing focusing the attention on a phagocyte expressing both types of receptors. Biburger and colleagues addressed this important question by characterizing the cell type specific expression of activating and inhibitory Fc γ Rs [33]. Tissue resident macrophages of the liver, spleen, and lung abundantly expressed Fc γ Rs, as well as neutrophils and monocytes. Quite strikingly and consistent with data from human clinical trials, a removal of the spleen had no effect on the reduction of platelet numbers induced by IgG1 and IgG2a switch variants of the 6A6 platelet specific autoantibody. Similar negative results were obtained in mice deficient or depleted for basophils, eosinophils, neutrophils, and inflammatory monocytes. Moreover, liver resident Kupffer cells did not colocalize with platelet immune complex deposits in the liver. Most surprisingly, autoantibody mediated platelet depletion was severely impaired once blood resident monocytes were removed. This monocyte subset was described to have a patrolling behavior in mice and humans and to have a high phagocytic activity [33, 65–67]. In line with the data from Fc γ R knockout mice, resident monocytes did express all activating Fc-receptors and the inhibitory Fc γ RIIB (Figure 2). The relevance of this observation for the human system is underlined by studies demonstrating that autoimmune patients receiving glucocorticosteroids show a marked and long lasting reduction of the corresponding human monocyte subset [68, 69]. As most of the animal studies have been performed with passive models of ITP, further studies will be necessary to elucidate the contribution of other cells of the mononuclear phagocyte system under clinically more relevant chronic forms of the disease. A role for resident monocytes under chronic inflammatory conditions has been suggested by studies showing that this monocyte subset is

FIGURE 2: Effector pathway of IgG dependent B cell depletion and platelet phagocytosis. Upon binding of antibodies to B cells or to thrombocytes, resident monocytes characterized by low expression of Ly6C and high expression of FcγRIV are responsible for B cell and platelet removal.

expanded and hyperactive in SLE-prone BXSB mice and in human patients having complications after heart surgery [70–72].

3.2. Autoimmune Hemolytic Anemia. In AIHA, autoantibodies trigger the removal of red blood cells resulting in a severe anemia of affected patients. Early studies using rabbit derived mouse red blood cell specific IgG antibodies demonstrated the crucial role of FcγRs in red blood cell removal [40, 42]. Later, more detailed studies performed by Izui and colleagues made use of clinically more relevant red blood cell specific IgG subclass switch variants from autoantibodies naturally developing in NZB (New Zealand Black) mice, which spontaneously develop AIHA [73]. Similar to the results obtained in mouse models of ITP, different IgG subclass switch variants of mouse red blood cell specific antibodies were demonstrated to differ in their activity in vivo. Again IgG2a and IgG2b subclasses were the most active variants, having a twentyfold higher potency to remove red blood cells [46, 74, 75]. This correlates well with the aforementioned concept of the A/I ratio which closely predicts this increased level of activity [17]. The lower level of activity of the IgG1 subclass could be explained by the strong negative regulation through the inhibitory FcγRIIB and the weaker binding to FcγRIII. IgG3 antibodies directed against red blood cells became active only at fourfold higher doses compared to IgG2a and IgG2b and worked exclusively through the complement system [74]. With respect to the IgG subclass specific involvement of activating FcγRs several striking similarities but also some differences compared to the ITP model system were observed. As expected, IgG1 autoantibodies were solely dependent on the activating FcγRIII [74, 76]. The activity of IgG2a and IgG2b, however, was dependent on both FcγRIII and FcγRIV [77, 78]. With respect to the high affinity FcγRI an involvement only at high doses of the IgG2a autoantibody

was noted, consistent with the fact that this activating FcγR is saturated with serum IgG in the steady state and that high amounts of immune complexes are required to trigger this receptor despite its high affinity for IgG2a [78]. Due to the different contribution of the individual activating FcγRs to AIHA, one might expect a different effector cell population to be involved in red blood cell removal. Indeed, there is convincing evidence that erythrocytes are phagocytosed by liver resident macrophages, such as Kupffer cells, which also express all relevant activating FcγRs, although an involvement of resident monocytes has not been excluded. Due to the difficulty of isolating liver resident macrophages, it has proven difficult to accurately evaluate the expression level of the different FcγRs, but one may expect to find a higher level of FcγRIII and a lower level of FcγRIV expression compared to what has been observed on the resident monocyte subset, where FcγRIV is the dominant receptor.

3.3. Autoantibody Dependent Inflammatory Arthritis. In this section, we will focus on data obtained in a well-established and frequently studied passive model of serum transfer arthritis originally developed by Korganow et al. [79]. Here, largely IgG1 but also IgG2a and IgG2b autoantibodies directed against the autoantigen glucose-6-phosphate isomerase (GPI) are responsible for joint inflammation and bone destruction [45]. Inflammatory arthritis differs in many aspects from the two autoimmune diseases we have discussed before. Most forward, a polyclonal antibody preparation (serum from mice with arthritis) is used to induce the disease. Moreover, the target of the autoantibody is not a specific cell but rather the tissue in the joints and autoantibody binding simply results not only in phagocytosis but also in severe inflammation and recruitment of different effector cells, carrying a varying array of activating FcγRs. Thus, the absence of mast cells, neutrophils, and macrophages completely blocks disease development [80–83], although the absolute requirement of mast cells was questioned more recently [84]. Moreover, a codominant role of activating FcγRs and the alternative complement pathway has been shown to be essential for tissue inflammation [85]. Due to this complexity, it is useful to go through the process of autoantibody dependent joint inflammation in a stepwise process and discuss the involvement of effector cells and FcγRs expressed on these cell types.

Quite interestingly, as early as ten minutes after injection of the arthritogenic serum, an opening of the endothelium in the paws was observed [86]. This reaction was absent in mast cell and neutrophil deficient mice and was strictly dependent on the activating FcγRIII, which is expressed on both cell types. In a similar manner the infiltration of neutrophils into the joint, which occurs three to four days later, was almost fully dependent on FcγRIII as well [85]. In addition, a small but significant contribution of FcγRIV was noted, consistent with the presence of small amounts of IgG2a and IgG2b autoantibodies within the arthritis inducing serum [45]. By a series of elegant cell transfer studies into mast cell deficient mice, it was demonstrated that this early recruitment of neutrophils was dependent on the release of IL1 by mast cells,

FIGURE 3: Effector pathways involved in autoantibody mediated joint inflammation and bone destruction. Shown are the cell types involved in joint inflammation and tissue destruction during autoantibody induced inflammatory arthritis. Upon immune complex binding to mast cells and tissue resident macrophages, proinflammatory mediators are released, which result in the recruitment of neutrophils and monocytes. Whereas neutrophils are responsible for tissue inflammation and joint swelling, inflammatory monocytes recruited to the joint develop into osteoclasts which are responsible for bone and cartilage erosion. See text for further details.

triggered most likely via crosslinking of FcγRIII by immune complexes (Figure 3) [83]. As the recruitment of neutrophils was also blocked in mice depleted for macrophages, it seems likely that immune complexes did also reach tissue resident macrophages resulting in crosslinking of FcγRIII and FcγRIV, resulting in the release of oxygen radicals and proinflammatory cytokines further attracting neutrophils to the site of inflammation. Given the most recent result in a novel mast cell deficient mouse strain, one may expect a codominant role of mast cells and tissue resident macrophages in the process of neutrophil recruitment. Once neutrophils have become recruited to the inflamed joint, IgG1 as well as IgG2a and IgG2b immune complexes may bind to FcγRIII and FcγRIV on neutrophils directly, mediate their activation, and result in a further recruitment of innate immune effector cells. Of note, FcγRI, despite being expressed on tissue resident macrophages and being the high affinity receptor for IgG2a, was not involved in neutrophil recruitment and joint inflammation, again consistent with the notion that it

may be saturated with serum IgG2a and not be available for immune complex binding. In addition to the FcγR system, the complement pathway does play a major role in this process as a deficiency in the complement proteins B, C3, and C5 and the C5a-receptor also blocked joint inflammation [85]. Among these complement pathway components, especially the anaphylatoxin C5a has the capacity to activate mast cells and attract neutrophils to the site of inflammation via binding to the C5aR expressed on these innate cell subsets.

Apart from joint swelling and the recruitment of neutrophils, yet another severe pathological change can be observed in affected joints and this is a destruction of bone and cartilage tissue. The major cell type responsible for this bone phenotype is osteoclasts which develop de novo from myeloid precursor cells within the proinflammatory cytokine environment of the inflamed joint. Although it is widely believed that the process of osteoclastogenesis is exclusively guided by T-cell and fibroblast derived TNFα and RANK-ligand, more recent data strongly supports a role of FcγRs and

antibodies in osteoclast development during inflammation (Figure 3) [87–89]. Consistent with the expression of all activating and the inhibitory FcγRIIB on tissue macrophages in the spleen, lung, and liver, also osteoclasts, which can be considered as the bone resident macrophage subset, did express all FcγRs [87]. Moreover, crosslinking of FcγRs did enhance osteoclast development and an osteoclast specific deletion of FcγRIV did strongly impair osteoclast development in vivo and abolish bone and cartilage destruction.

Taken together, studying the passive serum transfer arthritis model over the last 12 years has elucidated a complex network of interactions of autoantibodies with innate immune effector cells. Most strikingly, immune complexes influence virtually every cell type involved in the course of chronic inflammation via binding to cellular FcγRs or via activation of the complement pathway which will further fuel inflammation via C5a.

4. How Therapeutic Antibodies Mediate Their Activity In Vivo: Lessons from B Cell Killing

In this final paragraph we will discuss how monoclonal antibodies used in the therapy of autoimmune diseases and cancer may mediate their activity. We will focus on one of the most broadly and successfully used target structure in the clinic, which is CD20 expressed on normal and malignant B cells. There are several CD20 specific human IgG1 antibodies which are frequently used in the clinic. These include Rituximab, Ofatumumab, and Tositumumab as some of the most prominent examples. CD20 specific antibodies are distinguished by their capacity to redistribute CD20 into lipid rafts and are hence grouped into type I or type II CD20 specific antibodies. Whereas Rituximab and Ofatumumab can redirect CD20 into lipid rafts and therefore belong to type I antibodies, Tositumumab cannot and therefore is a member of the type II group [90, 91]. In contrast to cancer therapy, where the malignant B cell clone is the target of anti-CD20 therapy, in autoimmune diseases such as rheumatoid arthritis, SLE, chronic inflammatory neurological, and autoantibody dependent skin blistering diseases, the autoreactive B cells, responsible for autoantibody and proinflammatory cytokine production, are the target [91–96]. In addition, depletion of B cells has proven to be an effective treatment to prevent transplant rejection by inhibiting the production of transplant specific antibodies [97].

Based on data from human clinical trials in which human lymphoma patients were treated with Rituximab, there is convincing evidence that FcγRs are involved in the therapeutic pathway. Thus, patients carrying allelic variants in the FcγRIIA and FcγRIIIA genes which result in a receptor with increased affinity for IgG1 were demonstrated to have better therapeutic outcomes than those with low affinity variants [98–100]. Similar observations were made for other IgG1 therapeutic antibodies including Herceptin, strongly supporting the notion that an optimal interaction of the therapeutic antibody with FcγRs can increase therapeutic success [101]. Fully consistent with these findings, several studies in mice using either CD19 or CD20 specific antibodies

reported that not complement mediated lysis of B cells but rather FcγR dependent antibody dependent cellular cytotoxicity reactions may be critical for B cell killing in vivo [33, 47, 102–105]. Of note, Uchida and colleagues were able to show that in in vitro B cell killing assays complement was very effective in inducing B cell death. In contrast in vivo, mice deficient in various complement pathway components were not impaired in B cell killing at all, emphasizing that in vivo studies are essential to obtain a valid picture [47]. Further along these lines, several studies demonstrated quite convincingly that complement rather inhibits than supports IgG dependent target cell killing. For example, patients with Clq alleles resulting in a lower level of expression of this first essential component of the classical pathway of complement activation responded better to Rituximab therapy [106–111]. Nonetheless, Ofatumumab, a CD20 specific antibodies optimized for complement activation, shows promising results in the therapy of Rituximab refractory human cancer [94, 95]. More studies in humanized mouse models will be necessary to understand the contribution of different effector pathways in humans.

With respect to the different activating FcγRs involved in B cell killing, similar results compared to the ones obtained for autoantibody activity were obtained. Thus, IgG1 activity was fully dependent on activating FcγRIII, and IgG2a activity seemed to be codependent on FcγRI and FcγRIV, whereas IgG2b activity was largely dependent on FcγRIV [33, 47, 102]. In contrast to the AIHA model, however, no major involvement of FcγRIII was noted. With respect to the potential effector cells involved in B cell depletion, this is very interesting as it is very widely accepted that natural killer (NK) cells may be the dominant cell type responsible for ADCC reactions. This concept is largely based on in vitro assays, in which NK cells or so called lymphokine activated killer (LAK) cells primed with a large dose of IL2 to achieve a high level of activation, are coinubated with target cells in combination with the therapeutic antibody. While this indeed will lead to target cell killing, this does not allow drawing a conclusion with respect to the relevance of NK or LAK cells for ADCC reactions in vivo. Research performed in in vivo systems over the last 10 years, in fact, strongly supports a different scenario. Firstly, NK cells express only one activating FcγR in mice (FcγRIII) and in humans (FcγRIIIA or CD16). Although FcγRIII can bind to most IgG subclasses, autoantibody and therapeutic antibody activity is only impaired if the IgG1 subclass is used in mice. The more potent IgG subclasses IgG2a and IgG2b are much more dependent on FcγRIV and FcγRI, which are not expressed on NK cells [13, 43]. Moreover, a detailed analysis on FcγRIII expression on murine NK cells revealed a surprisingly low expression level compared to monocytes and other innate immune effector cells [112]. Consistent with this, not a depletion of NK cells but rather the removal of the innate mononuclear phagocytic system via toxic liposomes prevents the B cell depleting activity of CD20 specific antibodies in the mouse [33, 47, 104]. Building on this observation, later studies identified the resident monocyte subset, which shows the highest expression of FcγRIV and FcγRI in the blood, as potential effector cells responsible for B cell depletion [33].

The mechanism involved in B cell depletion remains to be characterized, but may include the release of reactive oxygen radicals and phagocytosis. With respect to human therapy, this finding is of major interest as humans undergoing a B cell depleting antibody therapy are usually treated with corticosteroids to prevent side effects such as the release of proinflammatory cytokines. As we have discussed before, however, treatment with corticosteroids also reduces the number of resident monocytes in humans, which may reduce the efficacy of antibody therapy. Apart from the apparent role of FcγRs on the effector cells, an involvement of FcγRIIB expressed on the target B cell in modulating the activity of the therapeutic antibody has been revealed more recently. Thus, type I CD20 specific antibodies including Rituximab but not type II antibodies showed a pronounced downmodulation of CD20 upon antibody injection in vivo [104]. Surprisingly this reduction in expression was dependent on FcγRIIB, which has the capacity to bind to IgG1 Fc-fragment of the CD20 antibody. As FcγRIIB is also associated with lipid rafts, this may explain why this regulation only occurred for type I and not for type II antibodies. Furthermore, this FcγRIIB-dependent reduction in CD20 expression correlated with the clinical efficacy of Rituximab in lymphoma patients. Thus, patients with a high expression level of FcγRIIB on tumor cells responded less to therapy than those with a low expression level. Based on this concept and on the threshold set by FcγRIIB for innate immune effector cell activation, blocking antibodies directed against FcγRIIB or therapeutic antibodies with an enhanced capacity to bind to activating and simultaneously lower binding to the inhibitory FcγRIIB may have a dual benefit. Firstly, they may enhance the binding of the therapeutic antibody to activating FcγRs and shift the A/I ratio in favor of cell activation; second, they may prevent the therapeutic antibody to become internalized into B cells and thereby maximize the likelihood to interact with innate immune effector cells.

5. Summary and Conclusions

Research over the last twenty years has elucidated many of the molecular and cellular components of the pathway responsible for IgG activity in vivo. Thus, the interaction of IgG antibodies with cellular Fcγ-receptors is critical for the induction of effector functions such as proinflammatory cytokine release and cellular cytotoxicity. Based on these findings, many second generation therapeutic antibodies with enhanced binding to activating FcγRs have been generated and are in the final stages of clinical evaluations. With respect to the inhibition of autoantibody activity, small molecule inhibitors targeting the signaling pathways triggered by activating FcγRs show very promising results. Future efforts in studying IgG activity in vivo should focus on the use of mice with cell type specific deletion of individual FcγRs which will allow us to further understand the complex network of interactions of immune complexes with the innate immune system. Moreover, mice transgenic for all human FcγRs or so called humanized mice, transplanted with a human immune system, will provide us with novel insights into the function of

human FcγRs in vivo and allow for an even better preclinical evaluation of novel therapeutic antibodies.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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