Fc receptors: Cell activators of antibody functions

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ABSTRACT

At the onset of an infection early defense systems, such as complement, get into action. Specialized leukocytes (white blood cells) of the innate immune system, including monocytes, macrophages, and neutrophils also participate as a first line of defense against infections. These early responses are rapid but not very specific and are usually not enough to clear completely many infections. The adaptive immune system is also needed to finish the job against many microorganisms. Antibody molecules, produced during the adaptive immune response, are crucial for preventing recurrent infections. Although, IgG antibodies are essential for controlling infections, these molecules do not directly damage the microorganisms they recognize. Today, it is established that leukocytes of the innate immune system are responsible for the protective effects of these antibodies. IgG molecules bind to their cognate antigens and are in turn recognized by specific receptors (Fcγ receptors) on the membrane of leukocytes. Crosslinking these receptors on the surface of leukocytes leads to activation of several effector cell functions. These effector functions are geared toward the destruction of microbial pathogens and the induction of an inflammatory state that is beneficial during infections. However, in autoimmune diseases, antibodies can direct these effector functions against normal tissues and cause severe tissue damage. In recent years, several factors that can modulate the IgG-FcγR interaction have been elucidated. In this review, we describe the main types of Fcγ receptors, and our current view of how antibody variants interact with these receptors to initiate different cell responses. In addition, new findings on the signaling role of individual Fcγ receptors are also discussed.

Keywords: Immunoglobulin; Antibody; Immunoreceptor; Neutrophil; Macrophage

1. INTRODUCTION

At the onset of an infection by different types of microorganisms, including viruses, bacteria, fungi, and protozoa, early defense systems, such as constitutive expression of antimicrobial peptides, and activation of complement get into action. These systems are rapid but not particularly specific. Specialized leukocytes (white blood cells) of the innate immune system, including monocytes, macrophages, and neutrophils also participate as a first line of defense against infections. These leukocytes can bind some microbial molecules, termed danger- and pathogen-associated molecular patterns (DAMPs and PAMPs, respectively) via numerous receptors such as the Toll-like receptor family [1,2]. In this way, leukocytes recognize microorganisms directly and prevent a massive infection [3]. These early responses however, are usually not enough to clear completely many infections. The adaptive immune system is also needed to finish the job against many microorganisms. Antibody molecules, produced during the adaptive immune response, are crucial for preventing recurrent infections [4]. At the beginning of the adaptive response, antibodies belong to the IgM class. These antibodies present low affinity for microbial antigens, but they can easily activate the classical complement pathway. Complement deposited on microorganisms can induce phagocytosis via complement receptors [5,6], or it can induce bacterial lysis via the formation of the membrane attack complex [7]. At later times of the adaptive response, antibodies belong mainly to the IgG class. These antibodies are of higher affinity and of much greater specificity for their particular antigen. Thus, IgG antibodies are key for controlling many microorganisms, as demonstrated by immunodeficiency disorders,
with low production of this class of antibodies, in which there is increased susceptibility to microbial infections [4]. Although, IgG antibodies are essential for controlling infections, these molecules do not directly damage the microorganisms they recognize. Today, it is established that leukocytes of the innate immune system are responsible for the protective effects of these antibodies. IgG molecules bind to their cognate antigens via their two fragment antigen-binding (Fab) sites, and are in turn recognized by specific receptors on the membrane of leukocytes. These receptors bind the Fc (fragment crystallizable) domain of IgG; thus, they are named Fcγ receptors (FcγR) [8,9]. In this way, IgG antibodies are the bridge between the two arms of the immune system, bringing together the specificity of recognition of the adaptive immune system and the destructive potential of the cells of the innate immune system. Crosslinking these receptors on the surface of leukocytes leads to activation of several effector cell functions. Depending on the cell type, and also on the Fcγ receptor type, these functions include phagocytosis, cell degranulation, production of various cytokines and chemokines, antibody-dependent cell-mediated cytotoxicity (ADCC), and activation of genes [10]. These effector functions are geared toward the destruction of microbial pathogens and the induction of an inflammatory state that is beneficial during infections. However, in autoimmune diseases, antibodies can direct these effector functions against normal tissues and cause severe tissue damage [11,12]. It is then of great interest to understand how various FcγR are activated to induce these cellular functions. In recent years, several factors that can modulate the IgG-FcγR interaction have been described. These factors include, the particular IgG subclass [13] and the glycosylation pattern of the antibody [14]. In addition, other molecules, such as members of the pentraxin family can bind FcγR [15,16], and certain glycosylation variants of antibodies can bind other cell membrane receptors different from FcγR [17-19]. In this review, we describe the main types of Fcγ receptors, and our current view of how antibody variants interact with these receptors to initiate different cell responses. In addition, new findings on the signaling role of individual Fcγ receptors are also discussed.

2. Fcγ RECEPTORS

2.1. Structure

Antibodies represent an important bridge between the specificity of the adaptive immune system, and the highly destructive mechanisms of cells of the innate immune system. Antibodies bind to microorganisms via their antigen-binding sites, and to Fc receptors on the surface of leukocytes, via their carboxyl terminal Fc portion. Receptors for the Fc portion of various immunoglobulin (Ig) classes have been described [20]. Fc Receptors for IgG (FcγR), for IgE (FcεR), and for IgA (FcαR) are known [20]. Crosslinking of Fcγ receptors with their IgG antibody ligands triggers various functions in many cells of the immune system. These cell functions include phagocytosis, cell degranulation, production of various cytokines and chemokines, ADCC, and activation of genes [10,21].

Fcγ receptors are a family of glycoproteins, part of the IgG superfamily. They consist of an IgG binding α-subunit, that usually pairs with accessory γ chains, which are important for receptor signaling (Figure 1).

In humans, three classes of FcγR have been identified, FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16) (Figure 1A) [22]. FcγR are coded for by different genes and differ in their relative avidity for IgG, molecular structure, and cellular distribution. FcγRI α-subunit presents three Ig-like extracellular domains, and binds monomeric IgG [23]. In contrast, FcγRII and FcγRIII present two Ig-like extracellular domains, and bind only multimeric immune complexes. FcγRI, expressed on monocytes, macrophages, and interferon-γ (IFN-γ)-stimulated neu-

Figure 1. Fcγ Receptor family. (A) The human family of receptors for the Fc portion of immunoglobulin G (IgG) molecules comprises three members FcγRI, FcγRII, and FcγRIII. The IgG binding α-subunit in the high affinity FcγRI, possesses three immunoglobulin (Ig)-like extracellular domains. The α-subunit in the other low-affinity receptors presents only two Ig-like domains. Activating receptors contain an ITAM (immunoreceptor tyrosine-based activation motif) sequence within the α subunit (for FcγRIIA) or within the accessory γ and ζ chains. In contrast, FcγRIIB is an inhibitory receptor containing an ITIM (immunoreceptor tyrosine-based inhibition motif) sequence. FcγRIIB is also an activating receptor, which is bound to the cell membrane via a glycosylphosphatidylinositol (GPI) anchor; (B) The murine family of receptors for the Fc portion of immunoglobulin G molecules comprises four members FcγRI, FcγRIIB, FcγRIII, and FcγRIV. Activating receptors (FcγRI, FcγRII and FcγRIV) contain an ITAM sequence within the accessory γ chains; while the inhibitory receptor (FcγRIIB) contains an ITIM sequence.
trophils, is associated with a dimer of the common Fc receptor (FcR) gamma-chain (also named FeRγ chain). Each γ chain contains tyrosine residues that are phosphorylated upon receptor activation and become docking sites for other signaling molecules. These tyrosine residues are found within a common motif known as ITAM, for immunoreceptor tyrosine-based activation motif [24, 25]. There are several isoforms of FeRRII, derived from its three genes and from alternative splicing. FeRRII isoforms are distributed differently on hematopoietic cells. FeRRIIA and FeRRIIC are found mainly in phagocytic cells (neutrophils, monocytes, and macrophages), whereas FeRRIIB is expressed mainly in B lymphocytes [22]. FeRRIIB expression is inducible in phagocytic leukocytes, for the negative regulation of cell functions, such as phagocytosis [8,26]. The human FeRRIIA is a particular receptor that does not have associated FeRγ chains. FeRRIIA contains an ITAM in its cytoplasmic portion, while FeRRIIB has a different tyrosine-containing motif involved in negative signaling. This motif is known as ITIM, for immunoreceptor tyrosine-based inhibition motif [27]. FeRRII has two isoforms: FeRRIIIA is a receptor with a transmembrane portion and a cytoplasmic tail, associated with an ITAM-containing homodimer of FeRγ chains on macrophages, natural killer (NK) cells, and dendritic cells. FeRRIIA expressed on basophils and mast cells associates with a heterodimer of γ/ζ chains and an extra β chain (Figure 1A) [22,23]. FeRRIIB is present exclusively on neutrophils and it is a glycosylphosphatidylinositol (GPI)-linked receptor, lacking a cytoplasmic tail. No other subunits are known to associate with it, and its signaling mechanism remains unidentified (Figure 1A). It is also worth noting that human FeRRIIA and FeRRIIB are exclusive receptors that are not found in other species [28].

In mice, several Fc receptors have also been described (Figure 1B). These receptors are very similar, yet not identical, to the human FeRγ [8]. FeRRI is expressed on monocytes and macrophages, and it is also associated with a dimer of FeRγ chains, which contain the ITAM motifs involved in receptor signaling. FeRRII is a receptor with a transmembrane portion and a cytoplasmic tail, associated with a dimer of FeRγ chains, containing ITAMs. This receptor is closer to the human FeRRIIA, as revealed by the genetic structure of FeRs in various species [13]. FeRRIIV is also an activating receptor expressed together with a dimer of FeRγ chains [29], and it is closer to the human FeRRIIIB (Figure 1B). FeRRIIV may be the most relevant activating FeγR in mice, due to its ability to bind IgG2a and IgG2b with higher affinity [13]. FeRRIIB is the negative receptor containing an ITIM motif in its cytoplasmic tail. It is expressed mainly in B lymphocytes but also in phagocytic leukocytes and dendritic cells. FeRRIIB, described first in B lymphocytes, down regulates the activation signals from the B cell antigen receptor (BCR) to inhibit antibody production by the B cell [30]. This inhibitory receptor also helps to regulate initiation of other cell functions in phagocytic leukocytes and dendritic cells by creating, together with activating Feγ receptors, a threshold for cell activation [13,31].

2.2. Cell Expression of Feγ Receptors

Fcγ receptors are found on many cells of the immune system, including granulocytes such as neutrophils and eosinophils; phagocytes such as neutrophils, monocytes and macrophages; and lymphocytes such as natural killer cells and B cells [23]. The wide variety of cellular responses regulated by Fcγ receptors is consequently not surprising. Monocytes and macrophages express all types of activating Fcγ receptors, FcγRI, FcγRII, and FcγRIII in humans, and FcγRI, FcγRII, and FcγRIV in mice (Table 1). Murine neutrophils express FcγRII and, FcγRIV, whereas human neutrophils express FcγRIIA and FcγRIIB.

It is noteworthy to mention that human neutrophils have the two unique FeγR, not present in neutrophils of other species. Thus, care should be taken when analyzing data derived from mouse studies of neutrophil FeγR function, since conclusions may not necessarily apply to human neutrophils. NK cells exclusively express FcγRIIIA in humans and FcγRIII in mice. Dendritic cells (DCs) also express various FcγRs, while B lymphocytes express mainly the inhibitory FcγRIIB (Table 1). Some T lymphocyte populations have also been reported to express FcγRs [32-34], but the role for these receptors in T cell function or development remains unclear and requires further studies.

2.3. Fcγ Receptor Signaling

Crosslinking of activating Fcγ receptors with their IgG antibody ligands triggers various functions in many cells of the immune system. As mentioned before, all activating receptors contain ITAM motifs involved in receptor signaling. The exact activation mechanism is not completely clear, but at the initial molecular events involve activation of Src family kinases followed by activation of Syk (spleen tyrosine kinase) family kinases.

The model for the initial steps of activating FeγR signaling is as follows: Upon crosslinking, the receptor associates with lipid rafts. Lipid rafts are small regions of the plasma membrane that are enriched in cholesterol and sphingolipids [35]. There, the receptor co-localizes with Src kinases. These kinases phosphorylate tyrosines within the ITAM. Phosphorylated tyrosines then become docking sites for Syk. This kinase then phosphorylates multiple substrates, including phosphatidylinositol 3-kinase (PI 3-K), phospholipase Cγ (PLCγ), and the adap-
### Table 1. Fc gamma receptors.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Principal antibody ligand</th>
<th>Affinity for ligand</th>
<th>Cell distribution</th>
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<tbody>
<tr>
<td><strong>Human</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>FcγRI (CD64)</td>
<td>IgG1 and IgG3 &gt; IgG4 &gt; IgG2</td>
<td>High (Kd ~ 10^8 M(^{-1}))</td>
<td>Macrophages, Neutrophils, Eosinophils, Dendritic cells</td>
</tr>
<tr>
<td>FcγRIIA (CD32)</td>
<td>IgG1 &gt; IgG2 and IgG3 &gt; IgG4</td>
<td>Low (Kd ~ 2 × 10^6 M(^{-1}))</td>
<td>Macrophages, Neutrophils, Mast cells, Eosinophils, Platelets, Dendritic cells</td>
</tr>
<tr>
<td>FcγRIIB (CD32)</td>
<td>IgG1 &gt; IgG2 and IgG3 &gt; IgG4</td>
<td>Low (Kd ~ 2 × 10^6 M(^{-1}))</td>
<td>Macrophages, Neutrophils, Mast cells, Eosinophils, Dendritic cells, B Cells</td>
</tr>
<tr>
<td>FcγRIIIA (CD16A)</td>
<td>IgG1 and IgG3</td>
<td>Low (Kd ~ 5 × 10^6 M(^{-1}))</td>
<td>Macrophages, Mast cells, Basophils, NK cells, Dendritic cells</td>
</tr>
<tr>
<td>FcγRIIB (CD16B)</td>
<td>IgG and IgG3</td>
<td>Low (Kd ~ 2 × 10^7 M(^{-1}))</td>
<td>Neutrophils</td>
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<table>
<thead>
<tr>
<th>Receptor</th>
<th>Principal antibody ligand</th>
<th>Affinity for ligand</th>
<th>Cell distribution</th>
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<tbody>
<tr>
<td><strong>Mouse</strong></td>
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<tr>
<td>FcγRI</td>
<td>IgG2a</td>
<td>High Kd ~ 1.6 × 10^8 M(^{-1})</td>
<td>Monocytes, Macrophages, Dendritic cells</td>
</tr>
<tr>
<td>FcγRIIB</td>
<td>IgG1</td>
<td>Low Kd ~ 3.3 × 10^6 M(^{-1})</td>
<td>B Cells, Dendritic cells</td>
</tr>
<tr>
<td></td>
<td>IgG2a</td>
<td>Kd ~ 0.4 × 10^6 M(^{-1})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IgG2b</td>
<td>Kd ~ 2.2 × 10^6 M(^{-1})</td>
<td></td>
</tr>
<tr>
<td>FcγRIII</td>
<td>IgG1</td>
<td>Low Kd ~ 0.3 × 10^6 M(^{-1})</td>
<td>Monocytes, Macrophages, Neutrophils, NK cells, Dendritic cells</td>
</tr>
<tr>
<td></td>
<td>IgG2a</td>
<td>Kd ~ 0.7 × 10^6 M(^{-1})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IgG2b</td>
<td>Kd ~ 0.6 × 10^6 M(^{-1})</td>
<td></td>
</tr>
<tr>
<td>FcγRIV</td>
<td>IgG2a</td>
<td>Low Kd ~ 2.9 × 10^7 M(^{-1})</td>
<td>Monocytes, Macrophages, Neutrophils, Dendritic cells</td>
</tr>
<tr>
<td></td>
<td>IgG2b</td>
<td>Kd ~ 1.7 × 10^7 M(^{-1})</td>
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*Data from [64,65]; **Data from [29,61].

or molecules SLP76 (SH2-domain-containing leukocyte protein of 76 kDa) and LAT (linker for activation of T cells) [36] (Figure 2). These molecules organize and activate several signaling pathways, depending on the cell type, leading to particular cellular responses and to transcriptional changes (Figure 2). For example, SLP76 seems to be important for FcγR signaling in neutrophils [37], while it seems to be dispensable in macrophages and NK cells [38,39]. LAT is another adaptor that seems to participate in many FcγR-mediated functions (Figure 2). LAT was reported to be constitutively associated with the common FcγR chain in monocytes [40], and to be important for efficient phagocytosis in macrophages [40]. Clearly, further studies are needed to identify the particular adaptor used by each type of Fc receptor in the various leukocytes, and to connect this to a unique cell response.

The inhibitory FcγRIIB is the negative receptor containing an ITIM motif in its cytoplasmic tail instead of an ITAM sequence [41]. It was described first in B lymphocytes, where it down regulates the activation signals from the B cell antigen receptor (BCR) (Figure 3A) to inhibit antibody production by the B cell [30]. Contrary to the activating receptors that engage several kinases, this inhibitory receptor signals by activation of phosphatases. Inositol 5-phosphatase (SHIP1) is the main enzyme activated upon crosslinking of FcγRIIB and BCR (Figure 3B) [27]. This phosphatase binds via its SH2 domain, to the phosphorylated tyrosines within the ITIM sequence of FcγRIIB. SHIP1 transforms phosphatidylinositol-3,4,5-trisphosphate (PIP₃) the main product of PI 3-K, into phosphatidylinositol-3,4-biphosphate (PIP₂); pre-
Figure 2. Activating Fcγ receptor signaling. FcγR cross-linking by immunoglobulin (IgG) bound to antigen (Ag), induces activation of Src family kinases and Syk family kinases in lipid rafts. These enzymes associate with phosphorylated tyrosines in the ITAM sequences. Syk then phosphorylates enzymes such as phosphatidylinositol 3-kinase (PI 3-K), phospholipase Cγ (PLCγ), and the adaptor molecules SLP76 (SH2-domain-containing leukocyte protein of 76 kDa) and LAT (linker for activation of T cells). PI 3-K produces phosphatidylinositol-3,4,5-triphosphate (PIP3), which leads to activation of Akt and extracellular signal-regulated kinase (ERK). PLCγ produces inositoltrisphosphate (IP3), and diacylglycerol (DAG). These second messengers cause calcium release from the endoplasmic reticulum (ER), and activation of protein kinase C (PKC), respectively. PKC leads to activation of ERK. Vav activates GTPases of the Rho and Rac family, which are involved in the regulation of the actin cytoskeleton. Other enzymes such as Bruton’s tyrosine kinase (Btk) also activate the GTPase Rac to induce activation of nuclear factors such as JNK and NF-κB. P represents a phosphate group. IP3R, receptor for IP3.

2.4. Coexpression of Fcγ Receptors and Threshold for Cell Activation

As indicated above, different leukocytes express more than one activating FcγR (Table 1), and most of these cells also express at the same time the inhibitory FcγRIIB. NK cells are particular leukocytes in this respect, because they only express FcγRIIIA in humans and FcγRIII in mice. The coexpression of both activating and inhibitory FcγR results in simultaneous triggering of activating and inhibitory signal transduction pathways (Figure 4A). Thus, a particular cell will respond when the sum of activating and inhibiting signals reach a threshold of activation that is determined by the relative expression of both types of FcγR (Figure 4B). The importance of the inhibitory FcγRIIB in regulating many IgG-mediated responses in different leukocytes was made evident in FcγRIIB-deficient mice, which showed enhanced activity of many IgG-mediated cell responses including: phagocytosis, immune complex-mediated inflammation, IgG-mediated passive and active anaphylaxis, and IgE-mediated anaphylaxis [42,43]. They also showed enhanced dendritic cell maturation, and antigen presentation [44-46]. These results thus confirmed that FcγRIIB regulates initiation of cell functions by generating, together with activating Fcγ receptors, a threshold for cell activation [31, 47].

2.5. Genetic Structure and Polymorphisms

Analysis of FcγR genes in different species has identified
orthologous receptors between mice and humans. The similarities come from gene localization and also from sequence homology of the extracellular portion of the receptors [8,13]. Therefore, the high affinity receptors FcγRIA and FcRI, the low affinity receptors FcγRIIA and FcγRIII, and also the low affinity receptors FcγRIIIA and FcγRIV cluster in the same area of chromosome 1 (Figure 5). Similarly, the inhibitory FcγRIIB gene maps to the same chromosome region, both in mice and humans (Figure 5). In addition, the human FcγRIIA and mouse FcγRIII, as well as the human FcγRIIIA and mouse FcγRIV, present high sequence homology in their extracellular domains [13]. Despite this similarity, the receptors are not equivalent, since important differences have been detected between mice and human responses to IgG. For example, the human FcγRI binds the IgG1 and IgG3 subclasses with high affinity, while the mouse FcγRI only binds IgG2a with high affinity (Table 1). In addition, the mouse FcγRIV is also able to bind IgE, while the human FcγRIIIA is not [48,49].

In addition, there are several polymorphisms in the human FcγRII and FcγRIII. For FcγRIIA two allelic variants exist expressing either arginine or histidine at position 131 [50,51]. For FcγRIIIA also allelic variants exist expressing either valine or phenylalanine at position 158 [52,53]. Similarly, for FcγRIIB on neutrophils, two isoforms exist, the NA1 and NA2 allotypes [54]. These isoforms differ by five nucleotides and four amino acids, with NA2 containing two additional N-linked glycosylation sites. These differences affect the capacity of FcγRIIIB to interact with human IgG. Therefore, neutrophils from individuals who are homozygous for the NA1 allele have better phagocytosis of IgG-opsonized targets than do neutrophils from NA2-homozygous individuals [55,56]. These multiple FcγR and their allelic variants vary greatly in their affinity for different IgG classes [57]. Thus, a great interest exists to understand how different IgG molecules engage different FcγR to activate the multiple cell responses associated with antibodies Fcγ receptor signaling.

3. IgG-MEDIATED CELL FUNCTIONS

3.1. Fcγ Receptor Affinity for IgG

A single antibody molecule does not bind to Fcγ receptors. However, antigen-antibody complexes promote many low affinity interactions between FcγR on the surface of leukocytes and antibody complexes. The low affinity of antibodies for individual leukocyte FcγR prevents receptors from binding antibodies in the absence of antigen, thus reducing the chance of immune cell activation when there is not an infection. Immune complexes induce the crosslinking of FcγR to activate the many different antibody-mediated cell responses. Immune complexes are clearly of different kinds, since they are formed by different classes of antibodies, and in vivo studies have suggested that the different IgG classes have different activities. For example, IgG2b/c was better in eliminating B cells [58] and T cell lymphomas [59] than IgG1. Likewise, using class-switch variants of anti-erythrocyte antibodies it was found that IgG2a and IgG2b were better in mediating phagocytosis of opsonized erythrocytes than IgG1 and IgG3 [60,61]. Also, polioencephalomyelitis induced by infection with lactate dehydrogenase-elevating virus (LDV) was delayed much better by IgG2a anti-LDV antibodies than any other IgG class of anti-LDV antibodies [62]. In addition, the severity of glomerular inflammation was greater for IgG2a, followed by
IgG2b and finally IgG1 [63]. All these reports confirmed that different IgG classes mediate different cellular responses in vivo through Fcγ receptors.

Because, immune complexes of different kinds induce different cell responses, there has been a great interest in determining which type of IgG binds to which FcγR and what particular receptor is involved in mediating the activity of particular IgG classes. Early studies showed that there is a high affinity receptor for IgG (FcγRI), which binds preferably to IgG1 in humans and IgG2a in mice [64,65]. This receptor is saturated with serum IgG on leukocytes in the blood. As mentioned before, the other receptors have only low affinity and can bind to IgG only in the form of immune complexes (Table 1) [64,65]. In addition, it was clearly established that most FcγR have a binding preference for IgG1 and IgG3 over the other classes of IgG (Table 1). Similarly, in mice it was found that IgG1 binds only to FcγRIII, while IgG2a binds to all types of activating FcγR, and IgG2b binds to FcγRII and FcγRIV. IgG3 does not seem to bind significantly to any of the FcγR (Table 1) [29,61]. In agreement with these data, IgG1 activity was lost in mice deficient in FcγRII [61,66]. For IgG2a and IgG2b, however the correlation with particular Fcγ receptors is not as simple. In some model systems the activity of these IgG classes was lost in FcγRIII-deficient mice, while it was not in others [13]. In a model of autoimmune hemolytic anemia, IgG2a-mediated response was highly dependent on FcγRIII, but also FcγRI and FcγRIV contributed to the development of severe anemia [67]. In another model of arthritis, mice deficient in FcγRI showed reduced cartilage destruction, and impaired protection from a bacterial infection, indicating the prominent role of FcγRI in IgG2a-dependent immune functions [68]. Thus, IgG2a used all activating Fcγ receptors with important contribution from FcγRI and FcγRII. Similarly, for IgG2b a particular interaction with a particular Fcγ receptor cannot be clearly established. In models of IgG2b-dependent B cell depletion [69], and nephrotoxic nephritis [70], inhibition of FcγRIV prevented B cell destruction and kidney inflammation, suggesting a central role for FcγRIV in IgG2b-mediated functions. However, in models of IgG2b-dependent autoimmune hemolytic anemia [67], acute glomerular inflammation [63], or acute lung injury [71], FcγRIV and also FcγRIII were important for the activity of this IgG class. Thus, an order of activity is observed among the different IgG classes, and a preference of engagement with particular Fcγ receptors.

Part of the mechanism used to create this IgG-FcγR selectivity is revealed by studies that measured the affinities of IgG classes toward both activating Fcγ receptors and the inhibitory FcγRIIB [13]. In this way, it was found that IgG1 has higher affinity for the inhibitory FcγRIIB than for the activating FcγRIII (Figure 6A), generating a high threshold for activation. In contrast, IgG2a and IgG2b have higher affinity for the activating FcγRIV than for the inhibitory FcγRIIB (Figure 6B), generating a lower threshold for activation. Thus, certain classes of IgG, such as IgG1, are more dependent on signaling from the inhibitory receptor. In agreement with this view, deletion of the inhibitory FcγRIIB increased IgG1 activity in models of platelet depletion and tumor cell killing [61]. It is important to note that this model for IgG-FcγR selectivity is not static and can be modulated by other factors, such as the pattern of FcγR expression on the different leukocytes (Table 1), and cytokines that can modify FcγR expression (Figure 7). Thus, Th1-type cytokines such as interferon-γ, and the anaphylatoxin C5a upregulate activating FcγRs expression and down-regulate FcγRIIB expression [72,73], whereas Th2-type cytokines, such as interleukin (IL)-4, IL-10, and transforming growth factor-beta (TGF-β) upregulate FcγRIIB expression [8,74].

3.2. Each FcγR Initiates Particular Signaling Pathways That Lead to Unique Cell Responses

All the reports previously described have confirmed that different IgG classes mediate different cellular responses in vivo by engaging particular Fcγ receptors depending on the relative affinity of these receptors for a particular.
Interestingly, the FcγERK in the nucleus, and also efficient phosphorylation of the nuclear factor Elk-1. The FcγRIIB signaling pathway resembles the classical ITAM-mediated pathway (Figure 2) [5], while FcγRIIIB signaling pathway remains a mystery (Figure 8). Taken together, these reports strongly support the hypothesis that each FcγR is capable of initiating particular signaling pathways that lead to unique cell responses.

4. EFFECT OF ANTIBODY GLYCOSYLATION ON Fc RECEPTOR FUNCTION

All antibodies are glycoproteins with various carbohydrate side chains attached to the protein backbone. The immunoglobulin classes IgM, IgA, and IgE have several exposed carbohydrate side chains. In contrast, IgG molecules have one carbohydrate side chain. This carbohydrate (sugar) side chain is important for IgG function. Deletion of the sugar side chain results in an altered conformation of the antibody molecule and in deficient binding to Fcγ receptors [80]. This carbohydrate domain is heterogeneous in its sugar composition. More than 30 different glycosylation variants of IgG can be found in serum of a healthy human or mouse individuals [14]. This heterogeneity is formed by variable addition of sugar residues such as sialic acid, N-acetylgalactosamine, galactose and fucose in straight or branching patterns. Although heterogeneous, the pattern of glycosylation seems to change in various physiological conditions. For example, terminal galactose and sialic acid residues were reduced in active autoimmune disease [18,81], while they were increased during pregnancy [82,83]. It is not clear what these changes in the glycosylation pattern
represent, but it seems that they can modulate IgG activity [84]. In contrast, IgG antibodies with reduced fucose residues presented higher affinity for human FeRγIIIA and its mouse ortholog FeRγRIV and showed improved antibody-dependent cellular toxicity against various tumor cells [61,85,86]. IgG antibodies with high levels of terminal sialic acid presented lower affinity for Feγ receptors and also reduced inflammatory activity [18,87,88]. In addition, IgG antibodies with abundant sialic can bind to other cellular receptors different from Fc receptors. SIGNR-1 (specific ICAM-3 grabbing nonintegrin related 1) and its human ortholog DC-SIGN (dendritic cell specific ICAM-3 grabbing nonintegrin) were identified as receptors for sialic acid rich IgG [87]. Moreover, this subpopulation of antibodies was also suggested to be responsible for the anti-inflammatory activity of intravenous Ig (IVIg) therapy, because in SIGNR-1 knockout mice, IVIg did not show an anti-inflammatory effect in a model of rheumatoid arthritis [87]. IVIg therapy that consists on administration of high doses of pooled serum IgG from many different donors has been used for many years to treat various autoimmune diseases such as rheumatoid arthritis and thrombocytopenia [19]. In addition, it has been reported that IVIg therapy can change the threshold for activation of cells by upregulation of the inhibitory FcRIIB and downregulation of activating FcγR in some mouse models and in patients with chronic inflammatory demyelinating polyneuropathy [70,89,90]. Thus, glycosylation patterns are critical for binding to particular Fcγ receptors and other novel antibody receptors. These reports underline the need of further studies on antibody-Fc receptor interactions to better understand the multiple effects of antibody molecules.

5. NEW LIGANDS FOR Fcγ RECEPTORS

Antibodies are the bona fide ligands for Fc receptors. However, some recent reports have identified other molecules different from IgG that can bind Fcγ receptors and can also activate the cell functions characteristic of antibodies. Two members of the pentraxin superfamily [91], which are multimeric cyclic proteins, are reported to bind human and mouse Fcγ receptors. These pentraxins are C-reactive protein (CRP) and serum amyloid P (SAP) [92-95]. These proteins are usually not found in serum of healthy individuals, but they are rapidly expressed in large amounts during inflammation and microbial infections. CRP and SAP are capable of binding to several microorganisms including bacteria and fungi and thus targeting them for phagocytosis by neutrophils and macrophages [92,96]. These reports suggest that these pentraxin proteins behave like antibodies recognizing foreign antigens on pathogens and directing them to cells of the innate immune system [16]. In support of this idea, it was also found that Fcγ receptor uptake of CRP-opsonized Streptococcus pneumoniae increased the immune response against these bacteria [95]. Moreover, CRP also seems to have an anti-inflammatory effect mediated by Fcγ receptors. In FeγR-deficient mice, administration of CRP did not protect from nephrotoxic nephritis or immune thrombocytopenia [94,97].

6. CONCLUSION

Fc Receptors expressed in a wide variety of leukocytes are capable of activating in response to various antibodies different cellular responses of great importance for host defense and for immune regulation. The different subclasses of IgG antibodies are recognized by Fcγ receptors with different affinities. Also singular Fcγ receptors seem to activate particular cell responses. This provides two ways for modulating cellular responses. In addition, expression of both activating and inhibitory Fcγ receptors establishes a threshold for activation of innate immune cells. Thus, Fcγ receptors are responsible of controlling the intensity of the immune response and of preventing unnecessary activation of innate immune cells, which might damage normal tissues. Novel glycosylation variants of IgG that bind Fcγ receptors with different affinities have been identified and also the anti-inflammatory activity of intravenous IgG therapy. Finally, novel receptors for antibody variants and novel Fcγ receptor ligands are been recognized. These new information together with new studies on IgG-Fcγ receptor interactions will certainly help us to develop new ways of controlling not only antibody-mediated effector functions directed against pathogens and tumors, but also the exaggerated antibody-mediated cell responses associated with auto-immunity.

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