Fcγ receptors as regulators of immune responses

Falk Nimmerjahn and Jeffrey V. Ravetch‡*

Abstract | In addition to their role in binding antigen, antibodies can regulate immune responses through interacting with Fc receptors (FcRs). In recent years, significant progress has been made in understanding the mechanisms that regulate the activity of IgG antibodies *in vivo*. In this Review, we discuss recent studies addressing the multifaceted roles of FcRs for IgG (FcγRs) in the immune system and how this knowledge could be translated into novel therapeutic strategies to treat human autoimmune, infectious or malignant diseases.

Regulatory T cells
A T cell subset that is capable of suppressing the activity of other antigen-specific T cells including autoreactive T cells. Depletion of regulatory T cells results in the loss of peripheral tolerance and the development of autoimmune disease.

**Laboratory of Experimental Immunology and Immunotherapy, Nikolaus-Fiebiger-Center for Molecular Medicine, University of Erlangen-Nuremberg, Glueckstr. 6, 91054 Erlangen, Germany.*
‡Laboratory for Molecular Genetics and Immunology, Rockefeller University, 1230 York Avenue, New York, New York 10021, USA.
e-mails: fnimmerj@molmed.uni-erlangen.de; ravetch@mail.rockefeller.edu
doi:10.1038/nri2206
Published online 7 December 2007

A productive immune response results from the effective integration of positive and negative signals that have an impact on both innate and adaptive immune cells. When positive signals dominate, cell activation and pro-inflammatory responses ensue, resulting in the elimination of pathogenic microorganisms and viruses. In the absence of such productive stimulation, cell activation is blocked and active anti-inflammatory responses can occur. Modulation of this binary system occurs through the action of cytokines, downstream signalling pathways and cell–cell contact. The perturbation of these thresholds can result in aberrant responses that are either insufficient to deal with pathogenic microorganisms or result in the loss of tolerance and the induction of autoimmune responses. The family of Fc receptors (FcRs) for IgG (FcγRs) provides a prime example of how simultaneous triggering of activating and inhibitory signalling pathways sets thresholds for cell activation and thus generates a well-balanced immune response[1]. Indeed, in a variety of human autoimmune diseases, such as arthritis and systemic lupus erythematosus (SLE), aberrant expression or the presence of allelic variants of FcγRs with altered functionality have been observed that contribute to the pathogenesis of these diseases[2]. In addition to their well-defined roles in triggering activation of innate effector cells, FcγRs function in antigen presentation and immune-complex-mediated maturation of dendritic cells (DCs), and in the regulation of B-cell activation and plasma-cell survival[3]. Thus, FcγRs not only control innate immune effector cell activation but are also involved in regulating the production and specificity of their ligands (that is, antibodies). Moreover, by regulating DC activity, FcγRs control whether an immunogenic or tolerogenic response is initiated after the recognition of antigenic peptides that are presented on the surface of DCs to cytotoxic T cells, T helper cells, and regulatory T cells. Thus, FcγRs are involved in regulating a multitude of innate and adaptive immune responses, which makes them attractive targets for the development of novel immunotherapeutic approaches (FIG. 1).

In this Review, we summarize our current understanding of the role of different members of the FcγR family in regulating immune responses. Our focus is largely based on results obtained in mouse knockout model systems and genetic linkage and association studies in human patients with autoimmune disease or cancer. Moreover, we address recent insights into how differential antibody glycosylation influences FcγR binding and the maintenance of an anti-inflammatory environment during the steady state. Finally, we will discuss how these results might be useful for improving human immunotherapy and how predictions can be tested in novel humanized mouse models.

The family of Fcγ receptors

To date, four different classes of FcγRs, known as FcγRI, FcγRIIB, FcγRIII and FcγRIV, have been recognized in mice (FIG. 2). Orthologous proteins corresponding to the mouse FcγRs have been identified in other mammalian species. In humans, monkeys and mice, most of the corresponding genes are clustered in close proximity on chromosome 1 next to the family of FcR homologous genes[4]. The human and primate FcγR systems are the most complex, as is exemplified by the existence of an FcγRI gene family (which includes *Fcgria*, *Fcgrib* and *Fcgric*) and an FcγRII gene family (which includes *Fcgriia* and *Fcgriic*) and the presence of several allelic FcγR variants[5] (FIG. 2). Based on the

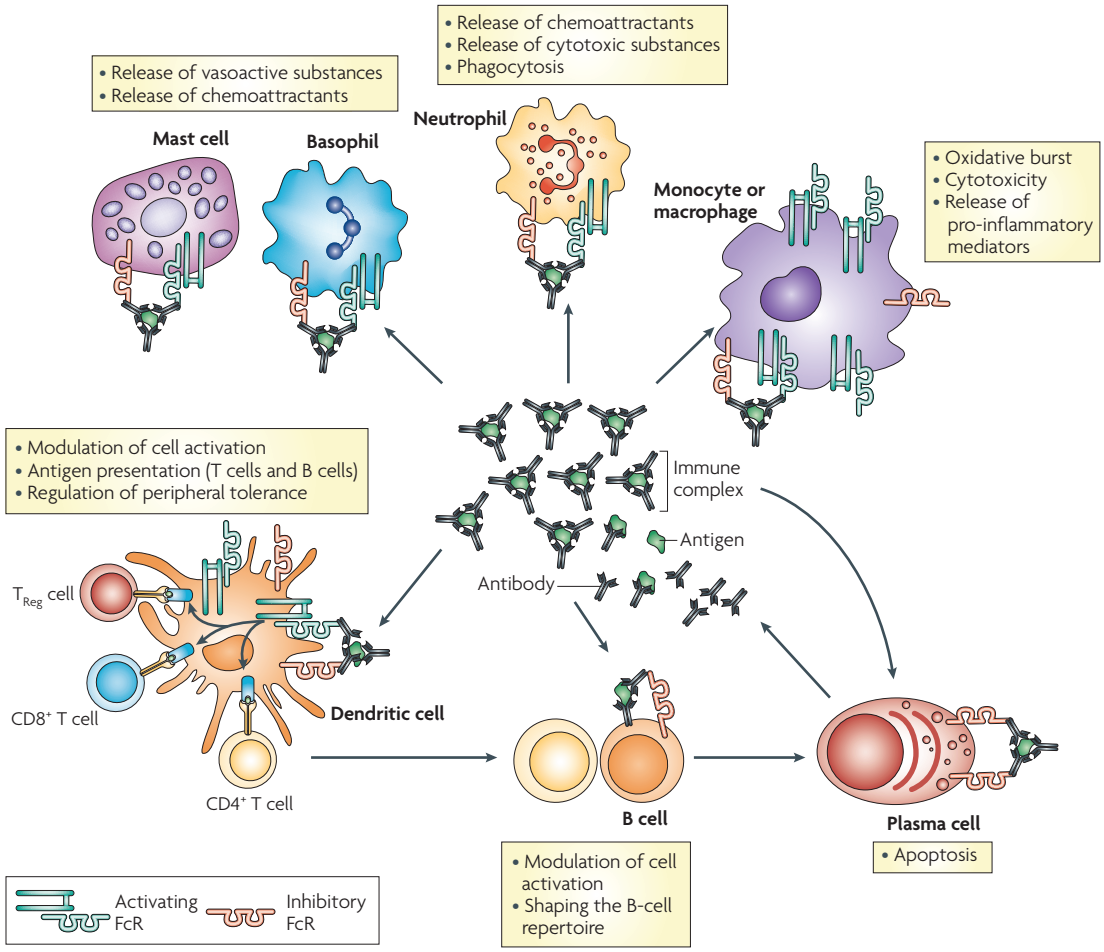

Figure 1 | **Regulatory functions of immune complexes.** Immune complexes bind to activating Fc receptors (FcR) and inhibitory FcRs that are expressed by innate immune effector cells such as basophils, mast cells, neutrophils, monocytes and macrophages, in which they trigger the indicated effector responses. Binding of immune complexes to FcRs on dendritic cells results in phagocytosis and presentation of antigenic peptides on MHC class I and class II molecules. Antigen-specific $CD8^+$ cytotoxic T cells, $CD4^+$ helper T cells or regulatory T cells (T_{Reg} cells) that recognize these peptide–MHC complexes become activated and mediate various effector functions such as killing of virus-infected cells, modulation of immune responses or providing T-cell help for antigen-specific B cells. B cells only express the inhibitory low-affinity FcR for IgG (FcγRIIB), which regulates activating signals transduced by the B-cell receptor (BCR). On plasma cells, which produce high levels of antigen-specific antibodies, BCR expression is very low or absent, resulting in exclusive triggering of inhibitory signalling pathways.

genomic localization and sequence similarity in the extracellular portion, mouse FcγRIV seems to be the orthologue of human FcγRIIIA, and mouse FcγRIII is most closely related to human FcγRIIA[4]. Despite these similarities, there are differences in the intracellular domains and the cellular expression pattern of these receptors that have to be taken into account when extrapolating data from animal studies to the human system. Human FcγRIIA, for example, contains an immunoreceptor tyrosine-based activation motif (ITAM) in its intracellular domain that is not present in mouse FcγRIII. In addition to the FcγRI and FcγRII gene families, there is a unique human receptor, known as FcγRIIIB, that is only expressed by neutrophils and that is attached to the cell membrane by a glycosyl-phosphatidylinositol (GPI) anchor. The affinity of the human FcγRs for the different IgG subclasses is significantly lower compared with their mouse counterparts. Nonetheless, results obtained in mouse model systems have been, and will continue to be, invaluable to decipher the role of FcγRs in the activity of the different antibody isotypes *in vivo*. Many of the basic principles and mechanisms underlying these activities have been identified in mice and have been recapitulated in humans[5].

The complexity in the FcγR family is mirrored by the presence of four different IgG subclasses in humans (IgG1–IgG4) and mice (IgG1, IgG2a, IgG2b and IgG3), which bind with varying affinity and specificity to different FcγR receptors[6,7]. In general, the number of IgG subclasses varies widely between different species, ranging from one subclass in rabbits to seven subclasses in horses, making it difficult to find orthologues. In humans, for example, IgG1 and IgG3 are the most pro-inflammatory

Immunoreceptor tyrosine-based activation motif (ITAM). A short peptide motif containing tyrosine residues that is found in the cytoplasmic tail of several signalling adaptor proteins such as the common γ- or CD3ζ-chain. It is necessary to recruit proteins that are involved in triggering activating signalling proteins. The consensus sequence is Tyr-X-X-(Leu/Ile)-X_{6-8}-Tyr-X-X-(Leu/Ile), where X denotes any amino acid.

IgG subclasses[2,6]. In mice, however, IgG2a and IgG2b are the most pro-inflammatory IgG molecules, and these show a greater activity than mouse IgG1 and IgG3 in many *in vivo* model systems[4].

FcγRs are widely expressed throughout the haematopoietic system[5]. Although initial studies suggested that certain T-cell subpopulations might express FcγRs, more recent evidence suggests that this is not the case[5,8–11]. The question of T-cell expression of FcγRs is, however, best considered to be an open one, as it is notoriously difficult to examine every possible subset or activation state of T cells for FcγR expression. Innate immune effector cells, such as monocytes, macrophages, DCs, basophils and mast cells express activating and inhibitory FcγRs. For example, in mice, monocytes and macrophages express all activating and inhibitory FcγRs (FcγRI–FcγRIV), neutrophils mainly express the inhibitory FcγRIIB and the activating FcγRIII and FcγRIV, whereas the expression of FcγRI, FcγRIIB and FcγRIII dominates on DCs[5]. There are two cell types that do not co-express activating and inhibitory receptors: NK cells solely express the activating receptor FcγRIII whereas B cells only express the inhibitory receptor FcγRIIB. FcγRIIB expressed by B cells functions as an important regulator of the activating signals that are transmitted by the B-cell receptor (BCR). Although the lack of inhibitory FcγR expression by NK cells suggests that these cells might be potent mediators of antibody-dependent cytotoxicity (ADCC) reactions, a growing body of experimental evidence in mouse model systems *in vivo* does not support this model. In addition to haematopoietic cells, FcγRs are expressed by follicular DCs (FDCs), endothelial cells, microglial cells, osteoclasts and mesangial cells. With the exception of FcγR expression by FDCs, the functional significance of FcγR expression on these other cell types has not been rigorously explored.

Traditionally, FcγR families are categorized according to the level of the receptor's affinity for specific IgG subclasses and the type of signalling pathway that it triggers — that is, whether it is inhibitory or activating[5,12] (FIG. 2). FcγRIIB is conserved in mice and humans and is the only known inhibitory FcγR; it transmits inhibitory signals through an immunoreceptor tyrosine-based inhibitory motif (ITIM) contained in its cytoplasmic region. All other receptors, with the exception of the human GPI-anchored FcγRIIIB, activate signalling pathways through ITAMs contained in their cytoplasmic regions. FcγRIA is the only

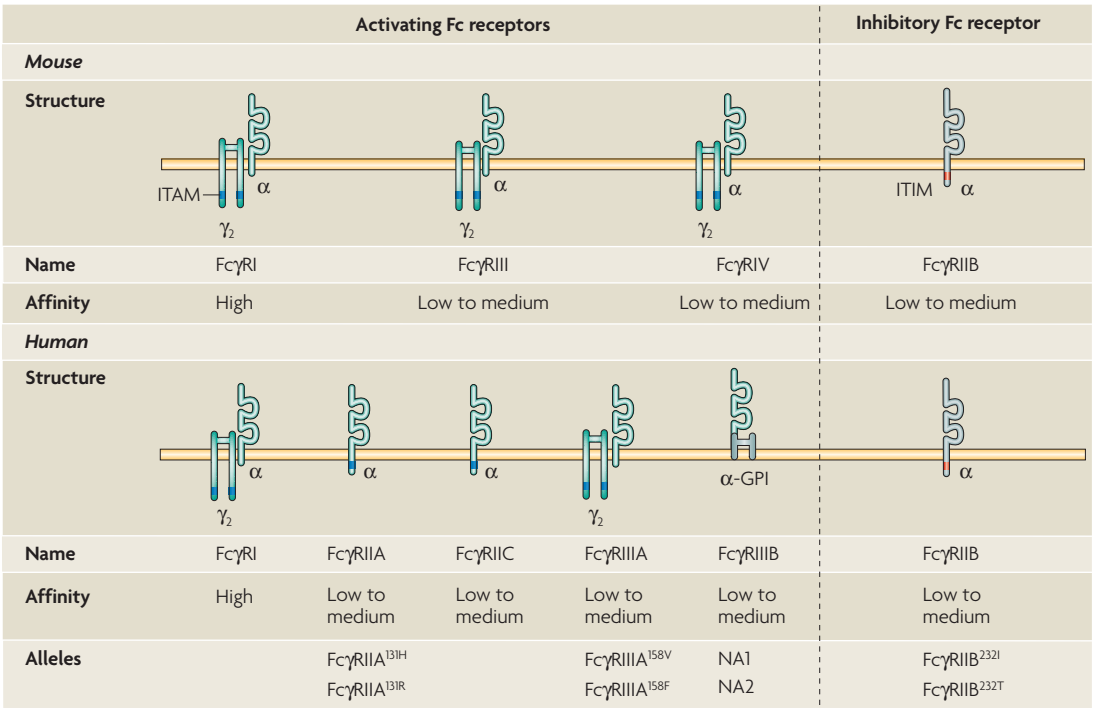

Figure 2 | **The family of Fc receptors for IgG.** Human and mouse Fc receptors for IgG (FcγRs) can be distinguished by their affinity for the antibody Fc-fragment and by the signalling pathways they induce. Mice and humans have one high-affinity receptor, FcγRI; all other FcRs have low to medium affinity for the antibody Fc fragment. With respect to the type of signals triggered by FcR crosslinking, there is one single-chain inhibitory receptor, FcγRIIB, which contains an immunoreceptor tyrosine based inhibitory motif (ITIM) in its cytoplasmic domain. With the exception of human FcγRIIA and FcγRIIC, an activating FcR usually consists of a ligand-binding α-chain and a signal-transducing γ-chain dimer, which carries immunoreceptor tyrosine based activating motifs (ITAMs). In addition, humans have a glycosyl-phosphatidylinositol (GPI)-linked receptor that is exclusively expressed by neutrophils, called FcγRIIIB, which exists in two allelic variants called neutrophil specific antigen 1 (NA1) and NA2. Moreover, a variety of human FcγR alleles with altered functionality exist. Thus, $Fc\gamma RIIA^{131H}$ and the $Fc\gamma RIIIA^{158V}$ have a higher affinity for certain IgG subclasses compared to their allelic counterparts. The $Fc\gamma RIIB^{232T}$ variant is unable to associate with lipid rafts and is therefore strongly impaired in its negative regulatory activity.

Immunoreceptor tyrosine-based inhibitory motif (ITIM). A short peptide motif containing a tyrosine residue that is found in the cytoplasmic portion of FcγRIIB and other regulatory proteins such as CD22 or CD72 that is necessary to recruit negative regulatory signalling proteins. The consensus sequence is (Ile/Val/Leu/Ser)-X-Tyr-X-X-(Leu/Val), where X denotes any amino acid.

known high-affininty receptor in mice and humans (the aforementioned human *Fcgrib* and *Fcgric* genes do not code for functional proteins); it binds IgG2a in mice or IgG1 and IgG3 in humans with an affinity of 10^8–10^9 M^{-1}. All other receptors have a 100–1000-fold lower affinity in the low to medium micromolar range and show a broader IgG subclass specificity[5]. For example, in mice, FcγRIIB and FcγRIII can bind to IgG1, IgG2a and IgG2b. By contrast, the medium-affinity FcγRIV selectively interacts with IgG2a and IgG2b[4,7]. Other isotypes, such as mouse IgG3, interact only very weakly with known FcγRs[6,7]. This low affinity of most of the FcγR proteins has an important function: it prevents binding to monomeric antibody molecules that are always present at high levels in the serum and thereby avoids the potential non-specific activation of pro-inflammatory responses. By contrast, the high-affinity FcγRI is constantly saturated with ligand. However, as has been described for the binding of IgE to the high-affinity FcεRI, cell activation only ensues after the receptors have been crosslinked by antigen[13,14]. Similarly, despite the capacity of certain IgG subclasses to bind to several low-affinity receptors *in vitro*, it has become clear that this does not necessarily imply that all of these receptors are involved in mediating the effector functions of a specific IgG subclass *in vivo*[15,16].

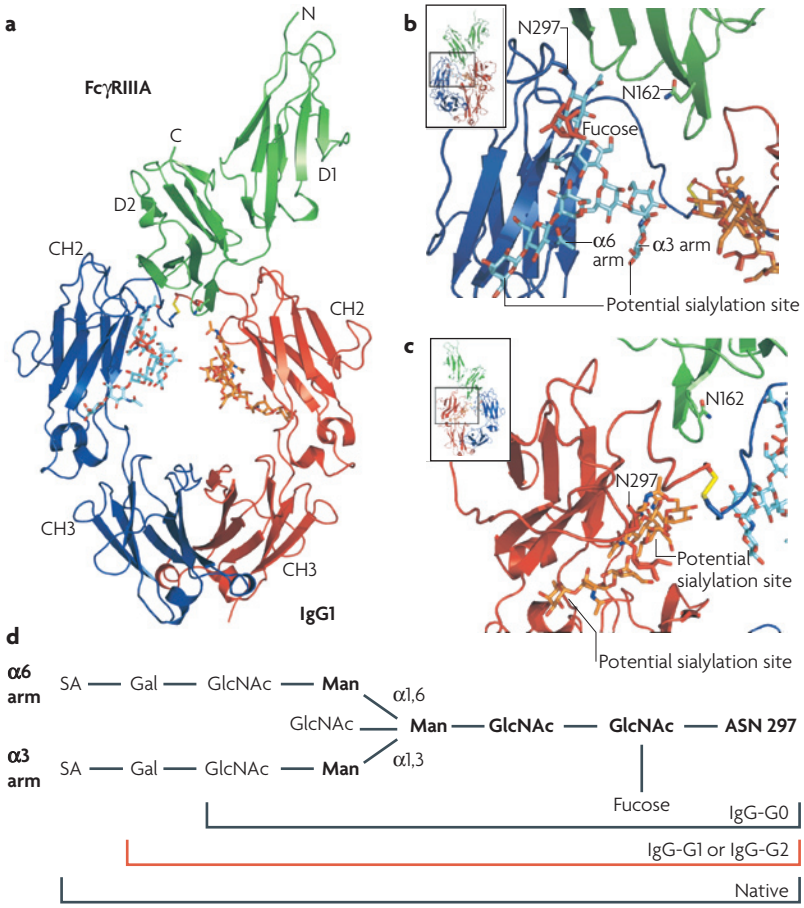

Figure 3 | **Antibody–FcγR interactions. a** | Crystal structure of the extracellular D1 and D2 domains of the low-affinity Fc receptor for IgG FcγRIIIA (in green ribbon representation) binding to the two heavy chains of human immunoglobulin G1 (IgG1). The CH2 and CH3 domains of the two IgG1 heavy chains are shown in red and blue ribbon representation. In addition, the two sugar moieties attached to the asparagine 297 (N297) residue of each of the CH2 domains of IgG1 are depicted in turquoise and orange stick representation. **b**,**c** | Enlarged view of the sugar moiety of both antibody heavy chains is shown. The N297 residue of the antibody heavy chain and the N162 residue of human FcγRIIIA, which contains an FcR-attached sugar moiety, are marked. The fully processed sugar structure of the antibody heavy chain is shown, with special emphasis on branching fucose (FUC) and sialic acid residues. The sialic-acid residues are shown as potential sialylation sites, as only a small portion of IgG molecules in serum contain one or two of these negatively charged sugar residues. The α3 and the α6 arms of the antibody sugar moiety are marked to demonstrate their differential orientation towards the antibody amino-acid backbone. **d** | The fully processed (native) antibody sugar moiety with all potential branching and terminal sugar residues. *In vivo*, only a minor fraction of IgG molecules have this sugar side chain. More frequently, partially processed moieties can be identified, such as IgG-G0 variants that lack all terminal sialic-acid and galactose residues. In IgG-G1 or IgG–G2 glycoforms, one or two galactose residues can be identified in the sugar side chain. Pictures of crystal structures (PDB entry: 1E4K) were generously provided by P. Sondermann (Roche).

Antibody–FcγR interactions

FcRs belong to the large immunoglobulin superfamily and are type I transmembrane glycoproteins (with the exception of the GPI-anchored FcγRIIIB). To precisely understand how FcRs interact with the antibody Fc fragment, determination of the individual and combined crystal structures of antibodies and FcRs was necessary. To date, crystal structures of the extracellular domains of several FcRs including FcγRIIA, FcγRIIB, FcγRIII and FcεRI have been solved[17–20]. Despite varying ligand specificity, the overall structure of different FcRs is closely related and can be superimposed with that of other family members with minimal aberrations[21]. The low-affinity receptors consist of two extracellular immunoglobulin-like domains (D1 and D2) that are bent at a 50–55° angle and are connected by a hinge region (FIG. 3). By contrast, the high-affinity receptor FcγRI contains an additional immunoglobulin-like domain (D3), which has been suggested to be important for high-affinity binding[22].

Structural information on antibody Fc fragments or intact antibodies alone or in complex with other proteins, such as protein A or protein G, rheumatoid factor or the neonatal FcR (FcRn), is available[23–28]. This information has revealed that the antibody Fc fragment, which consists of the CH2 and CH3 domains, acquires a horse-shoe-like structure with the sugar moieties attached to asparagine 297 (N297) residues in the CH2 domains protruding into the central cavity. The fully processed form of these sugar moieties is shown in FIG. 3d. In human serum IgG, however, more than 30 different variations consisting of partially processed variants can be found, introducing a high level of heterogeneity. In addition, the α3 and α6 arms have different orientations towards the antibody backbone and are frequently differentially processed in the same antibody molecule (FIG. 3).

Interestingly, variations in the composition of the sugar moiety can lead to subtle conformational changes in limited regions of the Fc fragment, as determined by the crystal structure of human IgG1 with or without fucose[29]. The breakthrough in the field was the solution of the co-crystal structure of human FcγRIIIA in complex with

the Fc portion of human IgG1 (REFS 30,31). Consistent with previous studies, the crystal structure suggests a 1:1 stoichiometry for the IgG1–FcγR interaction[32,33]. As shown in FIG. 3, only one domain of the FcγR makes contact with both lower hinge regions of the antibody Fc fragment and thereby it intercalates into the groove formed by the two different chains of the Fc fragment. Asymmetrical contacts are formed between the single FcγR molecule and the antibody Fc fragment, which precludes binding of a second FcγR molecule to the same Fc fragment. Therefore, solution of the co-crystal structure provided the molecular explanation of why crosslinking of FcγRs and cellular activation does not occur by binding of monomeric antibodies but depends on binding of multiple antibody Fc fragments present only in immune complexes, which are the physiological activators of innate immune effector cells[5]. This binding only slightly changes the structure of both the Fc fragment and the FcγR, with the Fc fragment loosing its dyad symmetry and the FcγR slightly increasing its interdomain angle[21]. In addition, the otherwise disordered antibody hinge region obtains an ordered structure in the co-crystal, suggesting that antibody–FcγR binding stabilizes the conformation of this region.

Activating FcγRs on innate immune effector cells

With the notable exception of human FcγRIIA and FcγRIIC, activating FcγRs cannot transduce activating signalling pathways autonomously. Thus, a functional activating FcγR consists of a ligand-binding α-chain and a signal-transducing adaptor molecule that contains ITAMs in its cytoplasmic domain (FIG. 2). Depending on the cell type, these adaptor proteins can differ. Whereas human FcγRIIIA is associated with the ITAM-containing CD3 ζ-chain in NK cells, it associates with the common γ-chain in monocytes and macrophages[5]. In basophils and mast cells, an additional β-chain, first described as a component of FcεR, is also associated with FcγRIII (REF. 34). In addition to its signalling function, γ-chain is also important for the assembly and cell-surface transport of the respective α-chains. Because of their expression on innate immune effector cells, activating FcγRs have been proposed to link the specificity of antibodies generated by the adaptive immune system to the potent effector functions of the innate immune system.

After crosslinking by immune complexes, the signalling pathways initiated by the different receptors are quite similar for different FcR classes and start with tyrosine phosphorylation of the ITAMs in the receptor-associated adaptor molecules by kinases of the SRC family[35,36] (FIG. 4). This leads to the recruitment of SYK-family kinases, followed by the activation of various downstream targets, such as the linker for activation of T cells (LAT), multimolecular adaptor complexes, and phosphoinositide 3-kinase (PI3K)[37–39]. By generating phosphatidylinositol-3,4,5-trisphosphate ($PtdIns(3,4,5)P_3$), PI3K creates membrane-docking sites for Bruton's tyrosine kinase (BTK) and phospholipase Cγ (PLCγ). Activation of PLCγ leads to an increased intracellular calcium level and triggering of further downstream signalling events[38,40]. Besides calcium-dependent pathways, the RAS–RAF–MAPK(mitogen-activated protein kinase)-pathway is of central importance for cell activation following FcγR crosslinking[5].

A crucial step in elucidating the important role of activating FcγRs was the generation of γ-chain-deficient mice[41]. The study of antibody activity in these mice demonstrated that the key function of activating FcγRs is to mediate IgG responses[41–45]. Antibody activity remained largely unchanged in mice deficient in a variety of the early components of the classical, alternative and mannose-binding lectin (MBL) pathway of the complement cascade, such as complement components C1q, C2, C3 and C4, MBL and CR2 (also known as CD21)[15,16,42,46–51]. By contrast, late components of the complement cascade, such as C5a, have been shown to be required in a model of autoimmune haemolytic anaemia (AIHA)[52,53]. Importantly, however, C5a did not participate in cytotoxic reactions directly but rather acted as a pro-inflammatory cytokine to upregulate activating FcγRs. In addition, generation of C5a was dependent on activating FcγRs and independent of known pathways of complement activation[52]. Further studies will be necessary to understand the precise mechanisms that lead to this FcγR-dependent generation of C5a. In general, however, the capacity of pro-inflammatory cytokines or mediators such as interferon-γ, tumour necrosis factor (TNF) or lipopolysaccharide to upregulate activating FcγRs is well established and further supports the important role of these proteins as mediators of antibody activity under such circumstances[4].

As mentioned before, studies on individual IgG subclasses revealed that despite the capacity of the low-affinity receptors to bind to several IgG subclasses *in vitro*, a more restricted set of activating FcγRs is involved in mediating the activity of the individual subclasses *in vivo*[15,16]. For example, in models of immunothrombocytopaenia (ITP), ADCC, and nephrotoxic nephritis, the activity of IgG2b antibodies was exclusively dependent on FcγRIV *in vivo*, despite its capacity to bind to both FcγRIII and FcγRIV *in vitro*[4,7,15,16,47]. In a similar manner, the activity of IgG2a was impaired in the presence of an FcγRIV blocking antibody in several model systems, but not in the absence of FcγRI and FcγRIII[15,16]. However, under some conditions, FcγRI and FcγRIII also contributed to the activity of the IgG2a subclass, suggesting that other factors such as anatomical location, cytokine milieu and effector-cell type have to be considered as well[54–57].

The only instance in which both the *in vitro* binding pattern and *in vivo* activity overlap is for mouse IgG1, which selectively binds FcγRIII. In model systems in which the IgG1 subclass is exclusively responsible for the phenotype, as for example in the K/BxN serum transfer arthritis model or in antibody-mediated tumour-cell, platelet or red-blood-cell depletion studies, animals deficient in FcγRIII showed abrogated antibody activity[15,16,56,58–60]. These findings also have some important implications with respect to the effector-cell types that are potentially responsible for these antibody activities *in vivo*. NK cells, for example, are widely believed to be the crucial mediators

Type I transmembrane glycoproteins
Glycoproteins of which the carboxyterminus of the polypeptide chain is located in the cytosol whereas the aminoterminus is exposed to the extracelluar space.

Neonatal FcR
(FcRn). FcRn is unrelated to classical FcRs and binds to a different region in the antibody Fc fragment. Structurally it is related to the family of MHC class I molecules and is responsible for regulating IgG half-life.

Dyad symmetry
The symmetrical arrangement of the repetitive structural elements an antibody molecule is composed of.

Complement cascade
There are three independent pathways that can lead to the activation of the complement cascade. The classical pathway is activated via C1q binding to immune complexes, the alternative pathway is triggered by direct C3 activation, and the lectin pathway is initiated by mannose-binding lectin (MBL) binding to the surface of microorganisms.

K/BxN serum transfer arthritis model
A mouse strain formed by crossing non-obese diabetic (NOD)/Lt mice with KRN T-cell-receptor-transgenic mice on the C57BL/6 background. As the KRN receptor on the T cells recognizes a peptide from the autoantigen glucose-6-phosphate isomerase, these mice develop an arthritis that is mediated, and transferable, by circulating antibody against glucose-6-phosphate isomerase.

of ADCC reactions *in vivo*. As mentioned before, these cells solely express FcγRIII and none of the other activating FcγRs. Although FcγRIII can bind mouse IgG1, IgG2a and IgG2b with a similar low affinity *in vitro*, the activity of IgG2a and IgG2b is not impaired in *Fcgriii*-knockout mice, in which NK cells are devoid of this activating FcγR[4]. Moreover, FcγRIV (and to a lesser extent, FcγRI), which have been demonstrated to mediate the activity of IgG2a and IgG2b subclasses *in vivo*, are not expressed on NK cells. Finally, it has been demonstrated in many model systems that the activity of IgG1 is negatively regulated by the inhibitory FcγRIIB. Thus, deletion of this receptor resulted in enhanced activity of the IgG1 subclass. A requirement for this negative regulatory

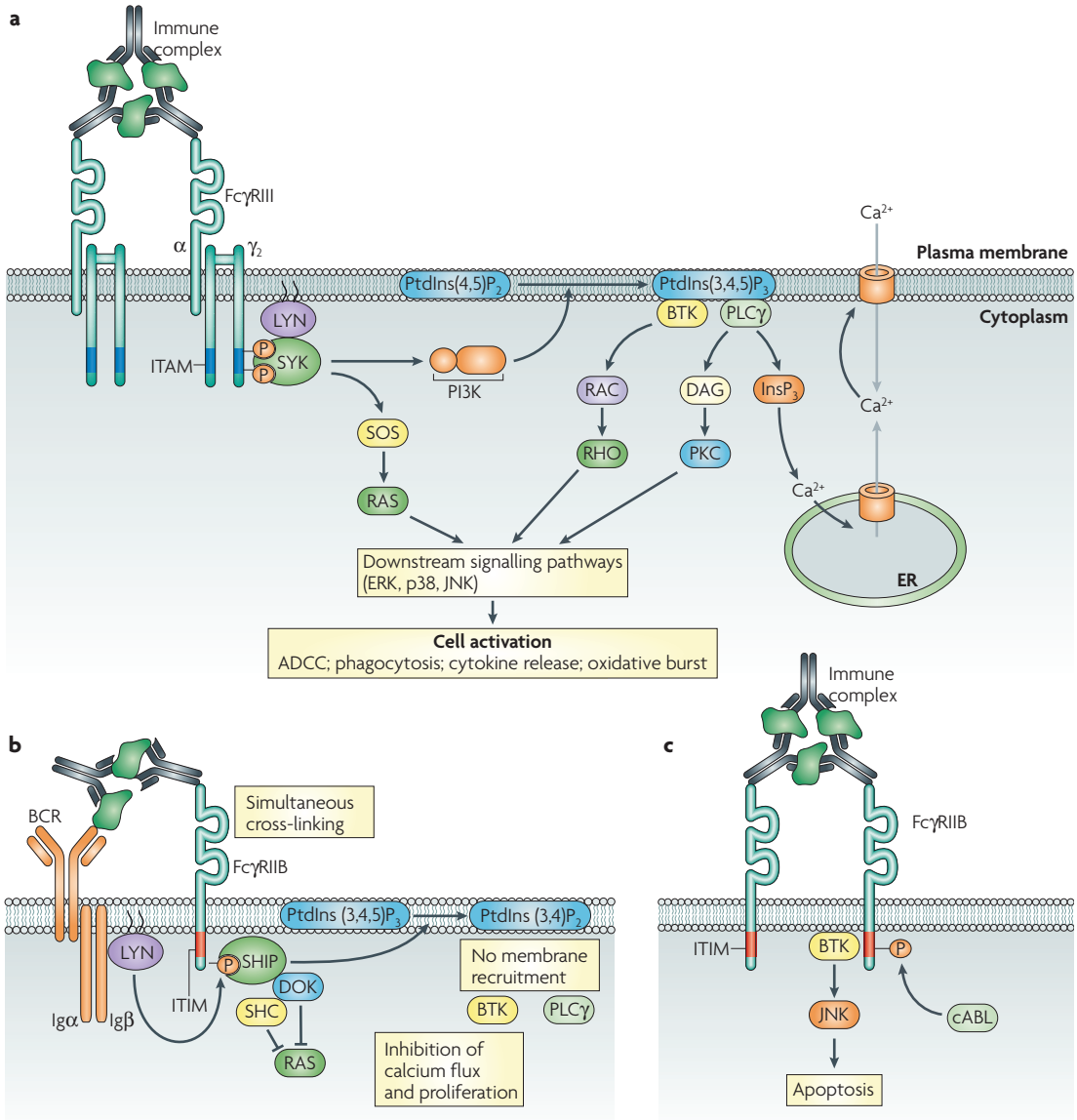

Figure 4 | **Signalling pathways triggered by activating and inhibitory FcγRs. a** | Crosslinking of activating Fc receptors for IgG (FcγRs) by immune complexes induces the phosphorylation of receptor-associated γ-chains by SRC kinase family members. This generates SRC homology 2 (SH2) docking sites for SYK, which in turn activates a number of other signal-transduction molecules such as phosphoinositide 3-kinase (PI3K) and son of sevenless homologue (SOS). The generation of phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) recruits Bruton's tyrosine kinase (BTK) and phospholipase Cγ (PLCγ), which leads to activation of downstream kinases and the release of calcium from the endoplasmic reticulum (ER). **b** | Simultaneous crosslinking of activating receptors like the B-cell receptor (BCR) and the inhibitory FcγRIIB leads to phosphorylation of the ITIM (immunoreceptor tyrosine-based inhibitory motif) in the cytoplasmic tail of FcγRIIB by LYN. This results in the recruitment of SRC-homology-2-domain-containing inositol-5-phosphatase (SHIP) and the hydrolysis of PtdIns(3,4,5)P_3 into phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2), which ultimately inhibits recruitment of pleckstrin homology (PH)-domain containing proteins such as BTK and PLCγ. **c** | Isolated triggering of FcγRIIB leads to B-cell apoptosis through ITIM-and SHIP-independent signalling pathways that involves the cABL kinase family, BTK and JUN N-terminal kinase (JNK). DAG, diacylglycerol; DOK, docking protein; $InsP_3$, inositol-1,4,5-trisphosphate; ITAM, immunoreceptor tyrosine-based activation motif; PKC, protein kinase C; RHO, RAS homologue; SHC, SH2-domain-containing transforming protein C.

function would be the co-expression of the inhibitory FcγR on the same cell that expresses the activating FcγRIII; NK cells, however, lack FcγRIIB expression. Taken together, the current *in vivo* evidence obtained in mouse model systems argues for granulocytes, monocytes or macrophages as the responsible cell types mediating IgG antibody activity. Definitive proof of this conclusion will require conditional deletion of the different activating FcγRs on selective effector cells, such as NK cells, macrophages, granulocytes and monocytes.

The extension of these results to human IgG and its cognate receptors was afforded by the introduction of therapeutic IgG1 antibodies for the treatment of neoplastic diseases. Several studies of lymphoma patients who had received Rituximab, a B-cell depleting chimeric IgG1 antibody that is specific for CD20, revealed that those patients with activating FcγR alleles that had a higher binding affinity for the Fc fragment of the therapeutic antibody displayed significantly improved clinical responses[61–63]. In addition, blocking FcγRIIIA in human patients with ITP significantly inhibited platelet depletion by human platelet-specific antibodies[64], thus validating the conclusions drawn from mouse model systems *in vivo*.

Another important function of activating FcγRs is their capacity to endocytose or phagocytose immune complexes, which includes antibody-coated microorganisms and soluble proteins[65,66]. In the case of granulocytes, monocytes and macrophages, this will mainly result in the rapid degradation of the engulfed material in lysosomal compartments. In the case of DCs, however, peptides derived from the phagosome or from endocytosed material are presented efficiently in the context of MHC class I and MHC class II proteins, which is a prerequisite for induction of an adaptive cellular immune response[67] (FIG. 1). The physiological importance of this FcγR-mediated uptake of antigens by DCs is demonstrated by the fact that antigens in the form of immune complexes prime a much stronger immune response than the antigen alone[67]. This process is modulated by the co-expression of the inhibitory FcγRIIB on these cells, which is discussed in the following section.

The regulatory function of the inhibitory FcγRIIB

The inhibitory FcγRIIB is the most broadly expressed FcγR, and is present on virtually all leukocytes with the exception of NK cells and T cells (FIG. 1). It is expressed in two different forms called FcγRIIB-1 and FcγRIIB-2. Whereas FcγRIIB-1 is exclusively expressed on B cells and has a low capacity to endocytose bound immune complexes, the FcγRIIB-2 form is expressed on all other cell types and is able to efficiently induce endocytosis or phagocytosis upon receptor crosslinking. Because of the broad expression pattern, it is not surprising that genetic deletion of this negative regulator results in complex phenotypic changes affecting innate and adaptive immune responses. Although cell-type-specific knockouts are not yet available, the use of different *in vivo* model systems permits predictions on the role of FcγRIIB expressed by different cell types.

FcγRIIB: A tolerance checkpoint for humoral immunity. Antibody binding to cellular FcγRs efficiently induces pro-inflammatory responses that lead to the removal of virus-infected or malignant cells, but it can also lead to the destruction of healthy tissues during autoimmune responses. Therefore, antibody specificity, as well as class switching to antibody isotypes that efficiently trigger pro-inflammatory reactions through their interaction with cellular FcγRs, have to be tightly controlled. Groundbreaking work over the last few years has established that several central and peripheral checkpoints exist throughout B-cell development to prevent the generation of autoreactive antibodies[68] (FIG. 5). On a molecular level, gene-deletion studies in mice have been instrumental in identifying several proteins (including the inhibitory FcγRIIB) that are involved in regulating B-cell activity.

One common theme that emerged from these studies is the importance of the ITIMs found in the cytoplasmic domains of these proteins[69,70]. Simultaneous triggering of ITIM-containing proteins with the BCR results in the recruitment of phosphatases such as SHIP (SH2-domain-containing inositol polyphosphate 5′ phosphatase) and SHP1 (SH2-domain-containing protein tyrosine phosphatase 1) that interfere with activating signalling pathways by hydrolysing phosphoinositide intermediates[3,71–73] (FIG. 4). This prevents the recruitment of pleckstrin homology (PH)-domain-containing kinases, such as BTK or PLCγ, to the cell membrane, thereby diminishing downstream events such as the increase in intracellular calcium levels. Thus, deletion of these regulatory proteins results in a lower threshold for B-cell activation and stronger activating signals after BCR crosslinking[71].

The importance of the inhibitory FcγRIIB in modulating B-cell activity and humoral tolerance is supported by studies of mice and humans (TABLE 1). Decreased or absent expression of FcγRIIB resulted in the development or exacerbation of autoimmune diseases and several mechanisms responsible for this reduced expression were identified. Regardless of the model system studied, FcγRIIB has emerged as a late checkpoint during peripheral B-cell development that acts at the level of class-switched B cells or antibody producing plasmablasts or plasma cells. Given the incomplete purging of autoreactive B cells from the immature repertoire in the bone marrow and the *de novo* generation of these cells during the process of affinity maturation, late peripheral checkpoints are of utmost importance.

Recent data suggests that the inhibitory FcγRIIB is important for regulating plasma-cell survival itself. The first evidence that the isolated triggering of FcγRIIB can induce apoptosis in B cells was reported 10 years ago. Co-engagement of the BCR and FcγRIIB in SHIP-deficient B cells induced apoptosis[72,73]. Similarly, the homo-oligomerization of FcγRIIB resulted in increased levels of B-cell death, and it was shown later that a signalling pathway dependent on BTK, JNK1 and cABL, but independent of SHIP and ITIM, was responsible for this phenotype[74,75]. It was suggested that this scenario might arise during the germinal-centre reaction, in

Class switching
If B cells recognize their cognate antigen in the spleen a portion of them switch the expression of their B-cell receptor from IgM to other isotypes such as IgG, IgA or IgE. The decision of which isotype is generated is strongly influenced by the specific cytokine milieu and other cells such as T-helper cells.

Pleckstrin homology (PH)-domain
An amino acid motif that enables proteins to recognize phosphatidylinositol-3,4,5-trisphosphate.

Plasmablasts
A short lived, dividing cell population that can develop from any type of activated B cell and that is characterized by its capacity to secrete antibodies.

Plasma cells
Terminally differentiated quiescent B cells that develop from plasmablasts and are characterized by their capacity to secrete large amounts of antibodies.

Affinity maturation
A process in which random mutations are introduced into the variable regions of the B-cell receptor genes followed by selection of cells with a higher affinity for the cognate antigen. This process takes place in specialized compartments of the spleen, which are known as germinal centres.

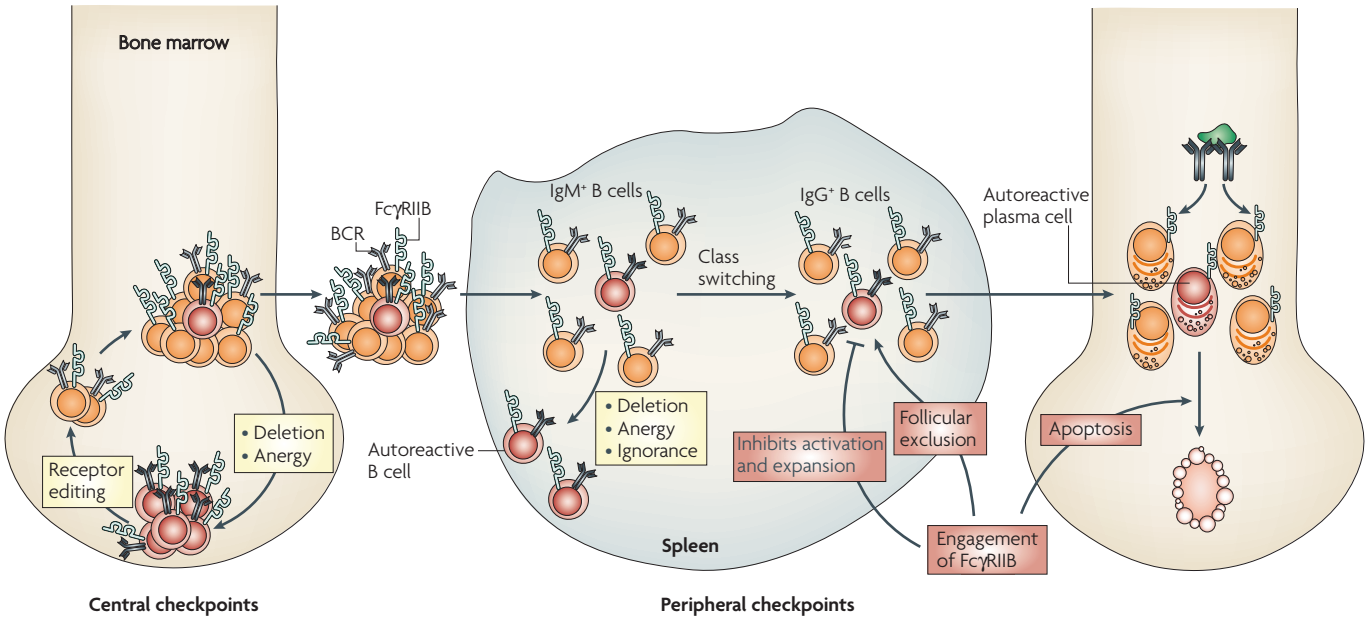

Figure 5 | **FcγRIIB as a checkpoint of humoral tolerance.** Autoreactive B cells (shown in red) that arise during the random rearrangement of antibody gene segments in developing B cells in the bone marrow or during the process of somatic hypermutation and affinity maturation in the spleen are controlled by several pathways, including receptor editing, clonal deletion or anergy induction. The inhibitory low-affinity Fc receptor for IgG (FcγRIIB) operates at several stages during later peripheral B-cell development. This receptor has been shown to be important for the follicular exclusion of low-affinity autoreactive B cells, for preventing B cells with a higher-affinity self-reactive receptor from becoming IgG positive plasma cells, and for triggering apoptosis of plasma cells upon immune complex binding, which is potentially important for regulating plasma-cell homeostasis during an immune response and deleting autoreactive plasma cells. BCR, B-cell receptor.

which B cells are in close contact with immune complexes presented on the surface of FDCs. Whereas B cells that generate a higher-affinity BCR will receive signals from both the BCR and FcγRIIB, B cells that lose affinity for the cognate antigen will only receive signals through FcγRIIB and will therefore be deleted.

Another situation in which a B cell expresses virtually no BCR and high levels of FcγRIIB is the terminally differentiated plasma cell. Plasma cells reside predominantly in niches in the bone marrow, where they have to receive survival signals from stromal cells[76]. If deprived of these anti-apoptotic signals, plasma cells rapidly die owing to pro-apoptotic signals triggered by a constant endoplasmic-reticulum-stress response induced by the continuous production of antibodies. One current conundrum is how the limited number of niches available in the bone marrow can accommodate the vast number of antigen-specific plasma cells that are necessary to protect the body from all types of pathogens[76]. How newly generated plasma cells gain access to these niches has remained a matter of debate, and models such as competitive dislocation have been proposed to explain this problem[77]. The pro-apoptotic signals triggered by isolated FcγRIIB crosslinking by immune complexes on plasma cells might be another elegant solution to this problem[78,79] (FIG. 5). Immune complexes generated *de novo* during an immune response could bind to plasma cells in the bone marrow and induce apoptosis on a fraction of cells, thus making space for newly generated plasma cells. Indeed, secondary immunizations with a new antigen result in reduced numbers of bone marrow plasma cells that are specific for the primary antigen[79].

Interestingly, plasma cells from autoimmune-prone mouse strains show absent or strongly reduced expression of FcγRIIB, and are resistant to induction of apoptosis. By contrast, restoration or overexpression of the inhibitory receptor could correct this defect[79]. Therefore, the failure to control plasma-cell persistence resulting from impaired FcγRIIB expression levels might account for their large number in autoimmune-prone mouse strains and ultimately for the development of chronic autoimmune disease. Correction of FcγRIIB expression levels might be a promising approach to interfere with autoimmune processes and to restore tolerance.

FcγRIIB as a regulator of adaptive immunity. DCs are the most potent antigen-presenting cells and can efficiently prime cellular immune responses. Besides this well-established function it is has become clear that during the steady state, these cells are actively involved in the maintenance of peripheral T-cell tolerance[72]. Thus, targeting antigens to DCs *in vivo* without the addition of co-stimulatory signals, such as those that trigger CD40 or Toll-like receptors, leads to the deletion or functional inactivation of antigen-specific $CD4^+$ and $CD8^+$ T cells[80–84]. This suggests that potentially self-reactive T cells that escaped deletion by central tolerance mechanisms in the thymus, will be rendered inactive upon recognition of self antigens on DCs in the periphery.

Competitive dislocation
This term refers to the competition of newly developed plasma cells for anatomical niches in the bone marrow that are already occupied by plasma cells that were generated during previous immune responses.

Cross presentation
The uptake of proteins by dendritic cells results in their degradation into small peptides in endosomal and lysosomal compartments and peptide presentation on MHC class II molecules. Cross presentation describes a process in which endocytosed material escapes into the cytoplasm where it is degraded by the proteasome followed by presentation on MHC class I molecules.

In addition, there is evidence that antigen presentation to $CD4^+$ T cells by DCs under tolerogenic conditions can induce regulatory T cells *de novo*[83]. Therefore, the maturation state of DCs has to be tightly controlled to prevent both the initiation of self-destructive responses and the generation of regulatory T cells during a protective antimicrobial immune response. A great number of activating and inhibitory cell-surface proteins involved in the regulation of DC activation have been identified[85]. Among them, the family of activating and inhibitory FcγRs has been shown to be of central importance for setting a threshold for DC activation and in the modulation of the adaptive cellular immune responses. This, however, is not the only function of FcγRs on DCs, as they are also important for endocytosis and/or phagocytosis of immune complexes and presentation of antigen-derived peptides on MHC molecules[86]. Therefore, FcγRs control three functions that are of central importance to any immune response initiated by DCs: antigen uptake, antigen presentation and cell activation.

As mentioned before, most FcγRs can only interact with antibodies in the form of immune complexes resulting in high-avidity binding. During an active immune response, a large number of immune complexes are generated owing to the priming of antigen-specific B cells (FIG. 1). Several studies have shown that immune complexes are potent activators of DCs and are able to prime stronger immune responses than antigen alone[67,87–90]. Importantly, FcγR-dependent immune-complex internalization not only resulted in MHC-class-II-restricted antigen presentation but also in cross-presentation on MHC class I molecules, thereby priming both CD4 and CD8 T-cell responses[67] (FIG. 1). The magnitude of this response is controlled by the inhibitory FcγRIIB, as DCs derived from *Fcgriib*-knockout mice generate stronger and longer-lasting immune responses *in vitro* and *in vivo*[91,92]. More importantly, FcγRIIB-deficient DCs or DCs incubated with a monoclonal antibody that blocks immune complex binding to FcγRIIB showed a spontaneous maturation[93,94]. This implies that the inhibitory FcγR not only regulates the magnitude of cell activation but also actively prevents spontaneous DC maturation under non-inflammatory steady-state conditions. Indeed, low levels of immune complexes can be identified in the serum of healthy individuals, emphasizing the importance of regulatory mechanisms that prevent unwanted DC activation[94].

In situations in which a maximal immune response is desirable, such as immunotherapy of malignancies or microbial infections, blocking FcγRIIB activity might be a novel way to obtain greater therapeutic efficacy[95]. Along these lines, it has been demonstrated that the genetic deletion of the gene encoding FcγRIIB results in the priming of more antigen-specific T cells[92]. Moreover, current approaches for targeting antigens to DCs *in vivo* by genetic fusion of the antigen to an antibody Fc fragment rely on an antibody mutant that does not bind to FcγRs to prevent FcγR-mediated modulation of cell activity. With the availability of antibody variants with enhanced binding to either activating or inhibitory FcγRs, however, it might become possible to integrate an additional activating or inhibitory second signal into the antibody–antigen fusion protein[96,97]. Depending on the application, this would permit the generation of either tolerogenic or immunogenic responses without adding secondary reagents. As will be discussed later, mouse models in which the mouse FcγRs have been replaced with their human counterparts (referred to as FcγR humanized mice) will be essential to test these optimized antibody variants *in vivo*.

FcγRIIB as a regulator of innate immunity. The inhibitory FcγRIIB is expressed on innate immune effector cells, such as mast cells, granulocytes and macrophages. As these cells have the capacity to trigger strong pro-inflammatory responses, their activation needs to be tightly controlled. In the case of antibody-mediated responses, such as phagocytosis, ADCC, allergic reactions and release of pro-inflammatory mediators, this is the function of the inhibitory FcγRIIB[5]. This crucial role is exemplified

Table 1 | **Involvement of FcγRIIB in autoimmune diseases**

Species	Type of impairment	Phenotype	Mechanism	Refs
Mouse (C57BL/6)	Gene knockout	SLE	Expansion of autoreactive IgG^+ plasma cells; impaired plasma-cell apoptosis; impaired follicular exclusion	119–12
		High susceptibility to induced autoimmue disease	Autoreactive B cells and higher activity of innate immune cells	79,123
Mouse (BALB/c)	Gene knockout	Higher susceptibility for induced SLE	Absent FcγRIIB expression	99,124
		Autoimmune hydronephrosis in PD1 and FcγRIIB double knockout	Dysregulation of B- and T-cell responses	98
Mouse (BXSB, NZM, NOD)	Promoter polymorphism	Autoimmunity (SLE, diabetes)	Reduced expression level	125–128
			Expanded plasmablast/plasma cell population	125–130
Human	Promoter polymorphism	Increased level of SLE; Increased disease severity	Low expression due to decreased binding of activating transcription factors	131–133
	Allelic variant (I232T)	SLE associated	Impaired recruitment to lipid rafts	134,135
	Not defined	SLE associated	Impaired expression of FcγRIIB	136

FcγRIIB, low-affinity Fc receptor for IgG; NOD, non-obese diabetic; NZM, New Zeland mixed; SLE, systemic lupus erythematosus; PD1, programmed cell death 1.

by enhanced macrophage responses in *Fcgriib*-knockout mice in models of collagen-induced arthritis and immune-complex-mediated alveolitis[98,99] (TABLE 1).

Interestingly, recent studies demonstrated that the inhibitory receptor contributes a varying level of negative regulation depending on the specific IgG subclass that is bound to the receptor[15]. This is consistent with the observation that different IgG subclasses have different activities *in vivo*. For example, in a variety of mouse model systems, IgG2a or IgG2b antibody subclasses are more active than IgG1 or IgG3 (REF. 4). Whereas IgG1 shows the strongest level of FcγRIIB-mediated negative regulation, the activity of IgG2a and IgG2b was increased less dramatically by the absence of this receptor[15]. This can be explained by the differences in the affinity of these isotypes for the different activating and inhibitory FcγRs. This ratio of affinities of a given IgG subclass for the activating versus the inhibitory receptor has been termed the A/I-ratio and it has emerged as a good predictive value for the activity of a specific IgG subclass *in vivo*[4,15]. These studies indicate that the effector cells responsible for mediating the activity of the different IgG subclasses express both activating and the inhibitory FcγRs. As NK cells lack FcγRIIB expression this argues against a role for NK cells as the responsible effector cell in mice *in vivo*. Indeed, myeloid cells that abundantly express FcγRIIB have been suggested to be the responsible effector-cell type in models of ADCC and SLE[51,100].

Antibody glycosylation and FcγR binding

The availability of structural information has allowed an understanding of which amino-acid side groups of antibodies and FcγRs directly interact or come into close contact on an atomic level. It has also become clear that, in addition to interactions between different amino acids, parts of the sugar moiety attached to N297 in the antibody Fc fragment also participate in this interaction (FIG. 3). This sugar side chain consists of a conserved biantennary heptasaccharide core structure containing mannose and N-acetylglucosamine (GlcNac) with varying additions of terminal and branching residues such as fucose, GlcNac, galactose and sialic acid[101]. Whereas the core sugar is highly ordered in the crystal structure, the terminal residues are more flexible. Deletion of the whole sugar moiety changes the structure of the antibody Fc fragment resulting in greatly impaired binding to cellular FcγRs[97,101]. By contrast, the interaction of deglycosylated IgG with FcRn is not affected, and this is consistent with other studies demonstrating that FcRn binds to a different region in the Fc portion.

Besides deglycosylated IgG, other more subtle variations in antibody glycosylation also have an impact on FcγR binding and antibody activity *in vivo*. Branching sugar residues such as N-acetylglucosamine or fucose have been implicated in affecting antibody activity *in vitro* and *in vivo*[15,102–104]. The lack of fucose increases the affinity of all IgG subclasses to human activating FcγRIIIA, and to its mouse orthologue FcγRIV, 10–50-fold. By contrast, binding to other human activating and inhibitory receptors, such as FcγRIIA or FcγRIIB, remains largely unchanged, suggesting that despite the overall similarity in protein structure, individual FcγRs have unique binding patterns. It is important to keep in mind that FcγRs as well as antibodies are glycoproteins and that the FcγR-associated sugar side chain or variations therein between different receptors might be of relevance. Indeed, it was demonstrated recently that the sugar moiety attached to the N162 residue in FcγRIIIA might be responsible for recognizing the presence or absence of branching fucose residues[102]. Whereas glycosylated FcγRIIIA had a higher affinity for Fc fragments without fucose, the same aglycosylated FcγR was unable to detect these differences[102]. Based on these data, a model was suggested in which the presence of fucose might sterically interfere with the antibody sugar moiety, resulting in a less stable interaction.

Another residue in the sugar moiety of the antibody Fc fragment that has been implicated in affecting antibody activity is galactose. Depending on the absence or presence of galactose, IgG glycovariants are either called IgG-G0 (no galactose) or IgG-G1 or IgG-G2 variants, containing one or two galactose residues (FIG. 3). It is known that humans with autoimmune disorders such as arthritis have an increased level of IgG antibodies without terminal galactose and sialic acid[101]. In addition, autoimmune-prone mouse strains such as MRL also have an increase in this specific IgG glycovariant, suggesting that the absence of these terminal sugar residues might be involved in autoantibody pathogenicity[105–107]. *In vitro* studies showed that the absence of galactose allows *de novo* binding of MBL, the first component of the lectin pathway of complement activation[108]. Thus, it was speculated that activation of the complement cascade might be responsible for the pathogenicity of these IgG-G0 glycovariants *in vivo*. More recent data, however, argues against the involvement of the lectin pathway of complement activation[109]. By using different IgG-G0 antibody subclasses and mouse models of autoimmunity, such as ITP or arthritis, it was demonstrated that these antibody glycovariants were not functionally impaired in mouse strains deficient in MBL. Similar results were obtained for downstream factors, such as the complement component C3 (REF. 109). By contrast, the activity of IgG-G0 as well as IgG-G1 and IgG-G2 glycovariants was abrogated in mouse strains deficient in all activating FcRs, suggesting that FcRs and not the complement pathway are responsible for IgG-G0 antibody activity *in vivo*.

Regardless of the role of galactose, the specific increase in IgG-G0 variants in mice and humans with autoimmune disease suggests that the terminal sugar residues of the antibody associated sugar moiety have some role in the regulation of antibody activity. Indeed, terminal sialic-acid residues have recently been demonstrated to be centrally involved in regulating antibody activity. In normal human serum and in purified serum IgG preparations such as intravenous immunoglobulin G (IVIG), a minor proportion of the IgG molecules carry terminal sialic-acid residues that are attached to the N297-linked sugar moiety[101]. By contrast, in other antibody isotypes

such as IgM, or on serum proteins such as transferrin, the majority of sugar moieties are fully processed and contain terminal sialic-acid residues. It has been suggested that structural constraints specific to the three dimensional arrangement of the sugar moiety in the IgG molecule, constrained within the groove formed by the two antibody heavy chains, prevent its complete processing. Interestingly, antibodies with site-directed mutations, such as D265A, in which acidic amino acids in the Fc fragment are exchanged with non-polar residues, have an increased level of terminal sialic acid suggesting that these negative charges in the antibody backbone do not favour additional negative charges in the sugar moiety that comes in close contact with these amino acids[101].

Evidence that this sugar residue is involved in immune regulatory processes was obtained by studying the mechanism of the anti-inflammatory activity of IVIG therapy[110]. IVIG therapy has proved to be an effective symptomatic treatment for acute and chronic inflammatory diseases such as ITP, SLE and rheumatoid arthritis[48]. Data from human clinical trials and a variety of mouse model systems clearly demonstrated that the Fc fragment contains most of the anti-inflammatory activity[47,110–113]. The high-dose requirement suggested that only a minor fraction of the IVIG preparation contains the active, anti-inflammatory component. In an effort to define if some of the more than 30 glycovariants are responsible for the therapeutic efficacy of IVIG, it was demonstrated that enriching the IVIG Fc fragment for sialic acid resulted in a greater than 10-fold enhancement of the anti-inflammatory activity[110]. Conversely, removal of sialic acid abrogated the therapeutic activity. Moreover, cytotoxic IgG antibodies enriched for sialic acid had a diminished activity *in vivo*, consistent with their reduced affinity for cellular FcγRs[110]. This suggests that the enhanced activity of this IVIG preparation is not dependent on direct binding to FcγRs, but rather indicates that other receptors exist that are able to sense sialic-acid-rich IgG[48]. Importantly, however, the anti-inflammatory activity of sialic-acid-rich IVIG was still crucially dependent on the presence and upregulation of the inhibitory FcγRIIB as has been demonstrated before in other mouse autoimmune models[47,111,113]. In other models systems a role for the activating FcγRIII in mediating IVIG-like effects was proposed[114,115]. Clearly, blocking the activating Fc receptor that is responsible for autoantibody activity will result in anti-inflammatory effects that resemble IVIG activity. Because of the low affinity of soluble IgG molecules to cellular FcγRs this seems very unlikely to be the actual mechanism of IVIG activity[48].

Combined with other studies showing that CSF1 (colony-stimulating factor 1)-dependent regulatory macrophages might be the cell population that is targeted by IVIG, a model was proposed in which sialic-acid-rich IVIG binds to an as yet unknown receptor on CSF1-dependent macrophages in the spleen with the capacity to recognize this IgG glycovariant[48,111]. Triggering of this receptor would induce the trans-upregulation of the inhibitory FcγRIIB on effector macrophages, thus raising the threshold for cell activation and the downmodulation of pro-inflammatory processes and tissue destruction. Further enhancing this effect, a simultaneous downmodulation of the responsible activating FcγR was observed in a model of nephrotoxic nephritis[47]. Taken together these results suggest that IgG-G0 serum antibodies do not gain functional activity, but it is rather the loss of the sialic-acid-rich IgG fraction that creates an environment favouring pro-inflammatory responses and the efficient interaction of autoantibodies with cellular FcγRs.

Future aspects

The family of FcγRs, owing to their central role in regulating humoral tolerance, antibody mediated effector functions and cellular immune responses, have emerged as central regulators for modulating both pro- and anti-inflammatory responses. The overwhelming evidence for the importance of the inhibitory FcγRIIB in controlling virtually all of these processes makes this a promising target for immunotherapy. Thus, restoring the function of FcγRIIB in human autoimmune diseases might be one novel therapeutic avenue for restoring tolerance in these patients. Indeed, this approach successfully prevented the development of autoimmune disease in several mouse strains prone to autoimmunity[116]. Similarly, the dominant role of activating FcγRs in mediating the pathogenic effects of autoantibodies suggests that blocking the interaction of immune complexes with specific FcγRs will interfere with chronic inflammatory processes. The reverse strategy might be useful to enhance ADCC reactions in immunotherapeutic settings such as antibody mediated destruction of malignant or virus-infected cells. Optimizing antibody activity by enhancing the interaction with activating FcγRs, or blocking antibody binding to the inhibitory receptor, is likely to improve the therapeutic outcome. Finally, the use of antibodies as targeting molecules to deliver antigens to DCs for antigen presentation to T cells might be optimized by using targeting antibody variants that specifically interact either with activating or inhibitory FcγRs on DCs, thereby providing either co-stimulatory or inhibitory signals.

The ability of antibodies to trigger innate effector cells renders them potentially dangerous molecules. Unwanted activation of these cell types might result in the massive release of pro-inflammatory cytokines leading to life-threatening complications. In instances in which the activity of an antibody does not require effector-cell activation, such as is the case for some blocking antibodies, variants that cannot interact with cellular FcγRs should be considered. Therefore, whether an antibody is engineered to enhance or inhibit specific FcγR interactions will depend on the clinical application. How are such engineered antibodies to be evaluated? As described above, *in vitro* systems fail to recapitulate the diversity and specificity of Fc–FcγR interaction. Animal models, be they rodent or non-human primate models, are inadequate, as exemplified by the catastrophic outcome of a recent clinical trial with a CD28-specific superagonistic antibody[117]. Therefore, new animal models that fully

recapitulate the human immune system more closely would be a great benefit. The development of such systems, however, presents formidable technical challenges. However, an intermediate tool that is within reach is the use of FcγR humanized mice. The feasibility of this approach has been demonstrated in recent years with the generation of the human FcγRIA, FcγRIIA, FcγRIIB, FcγRIIIA and FcγRIIIB transgenic mouse strains that reproduce the unique pattern of human gene expression[18,58,118]. Introduction of these transgenes into mouse strains deleted for their mouse counterparts has resulted in humanized FcγR animals which can now be used as a preclinical, *in vivo* platform for the evaluation of Fc-engineered human IgG or Fc-based therapeutics. When evaluating results from these mice it has to be kept in mind, however, that mouse serum IgG subclasses that can bind to human FcγRs are present in these animals.

The study of FcRs continues to be an exciting and clinically important field because of their obvious importance for human diseases and antibody based therapeutics. By advancing our knowledge in classical mouse models combined with novel humanized strains we should be able to gain the necessary information to translate our extensive knowledge in this field into novel and effective therapies for a variety of human diseases.

1. Ravetch, J. V. & Lanier, L. L. Immune inhibitory receptors. *Science* **290**, 84–89 (2000).
2. Nimmerjahn, F. Activating and inhibitory FcγRs in autoimmune disorders. *Springer Semin. Immunopathol.* **28**, 305–319 (2006).
3. Bolland, S. & Ravetch, J. V. Inhibitory pathways triggered by ITIM-containing receptors. *Adv. Immunol.* **72**, 149–177 (1999).
4. Nimmerjahn, F. & Ravetch, J. V. Fcγ receptors: old friends and new family members. *Immunity* **24**, 19–28 (2006).
5. Ravetch, J. V. in *Fundamental Immunology* (ed. Paul, W. E.) 685–700 (Lippincott-Raven, Philadelphia, 2003).
6. Dijstelbloem, H. M., van de Winkel, J. G. & Kallenberg, C. G. Inflammation in autoimmunity: receptors for IgG revisited. *Trends Immunol.* **22**, 510–516 (2001).
7. Nimmerjahn, F., Bruhns, P., Horiuchi, K. & Ravetch, J. V. FcγRIV: a novel FcR with distinct IgG subclass specificity. *Immunity* **23**, 41–51 (2005).
This study identifies FcγRIV as a functional activating FcR that is responsible for mediating the activity of IgG2a and IgG2b antibody subclasses *in vivo*.
8. Anderson, C. L. & Grey, H. M. Receptors for aggregated IgG on mouse lymphocytes: their presence on thymocytes, thymus-derived, and bone marrow-derived lymphocytes. *J. Exp. Med.* **139**, 1175–1188 (1974).
9. Leclerc, J. C., Plater, C. & Fridman, W. H. The role of the Fc receptor (FcR) of thymus-derived lymphocytes. I. Presence of FcR on cytotoxic lymphocytes and absence of direct role in cytotoxicity. *Eur. J. Immunol.* **7**, 543–548 (1977).
10. Stout, R. D. & Herzenberg, L. A. The Fc receptor on thymus-derived lymphocytes. I. Detection of a subpopulation of murine T lymphocytes bearing the Fc receptor. *J. Exp. Med.* **142**, 611–621 (1975).
11. Takai, T. Roles of Fc receptors in autoimmunity. *Nature Rev. Immunol.* **2**, 580–592 (2002).
12. Hulett, M. D. & Hogarth, P. M. Molecular basis of Fc receptor function. *Adv. Immunol.* **57**, 1–127 (1994).
13. Galli, S. J. *et al.* Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu. Rev. Immunol.* **23**, 749–786 (2005).
14. Gould, H. J. *et al.* The biology of IgE and the basis of allergic disease. *Annu. Rev. Immunol.* **21**, 579–628 (2003).
15. Nimmerjahn, F. & Ravetch, J. V. Divergent immunoglobulin G subclass activity through selective Fc receptor binding. *Science* **310**, 1510–1512 (2005).
16. Hamaguchi, Y., Xiu, Y., Komura, K., Nimmerjahn, F. & Tedder, T. F. Antibody isotype-specific engagement of Fcγ receptors regulates B lymphocyte depletion during CD20 immunotherapy. *J. Exp. Med.* **203**, 743–753 (2006).
17. Garman, S. C., Kinet, J. P. & Jardetzky, T. S. Crystal structure of the human high-affinity IgE receptor. *Cell* **95**, 951–961 (1998).
18. Maxwell, K. F. *et al.* Crystal structure of the human leukocyte Fc receptor, Fc γRIIa. *Nature Struct. Biol.* **6**, 437–442 (1999).
19. Sondermann, P., Huber, R. & Jacob, U. Crystal structure of the soluble form of the human Fcγ-receptor IIb: a new member of the immunoglobulin superfamily at 1.7 Å resolution. *EMBO J.* **18**, 1095–1103 (1999).
20. Sondermann, P., Kaiser, J. & Jacob, U. Molecular basis for immune complex recognition: a comparison of Fc-receptor structures. *J. Mol. Biol.* **309**, 737–749 (2001).
21. Radaev, S. & Sun, P. Recognition of immunoglobulins by Fcγ receptors. *Mol. Immunol.* **38**, 1073–1083 (2002).
22. Allen, J. M. & Seed, B. Isolation and expression of functional high-affinity Fc receptor complementary DNAs. *Science* **243**, 378–381 (1989).
23. Corper, A. L. *et al.* Structure of human IgM rheumatoid factor Fab bound to its autoantigen IgG Fc reveals a novel topology of antibody-antigen interaction. *Nature Struct. Biol.* **4**, 374–381 (1997).
24. Deisenhofer, J. Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from *Staphylococcus aureus* at 2.9- and 2.8-Å resolution. *Biochemistry* **20**, 2361–2370 (1981).
25. DeLano, W. L., Ultsch, M. H., de Vos, A. M. & Wells, J. A. Convergent solutions to binding at a protein–protein interface. *Science* **287**, 1279–1283 (2000).
26. Harris, L. J. *et al.* The three-dimensional structure of an intact monoclonal antibody for canine lymphoma. *Nature* **360**, 369–372 (1992).
27. Martin, W. L., West, A. P., Jr., Gan, L. & Bjorkman, P. J. Crystal structure at 2.8 Å of an FcRn/heterodimeric Fc complex: mechanism of pH-dependent binding. *Mol. Cell* **7**, 867–877 (2001).
28. Saphire, E. O. *et al.* Contrasting IgG structures reveal extreme asymmetry and flexibility. *J. Mol. Biol.* **319**, 9–18 (2002).
29. Matsumiya, S. *et al.* Structural comparison of fucosylated and nonfucosylated Fc fragments of human immunoglobulin G1. *J. Mol. Biol.* **368**, 767–779 (2007).
30. Radaev, S., Motyka, S., Fridman, W. H., Sautes-Fridman, C. & Sun, P. D. The structure of a human type III Fcγ receptor in complex with Fc. *J. Biol. Chem.* **276**, 16469–16477 (2001).
31. Sondermann, P., Huber, R., Oosthuizen, V. & Jacob, U. The 3.2-Å crystal structure of the human IgG1 Fc fragment-FcγRIII complex. *Nature* **406**, 267–273 (2000).
By generating a crystal structure of human FcγRIIIA in complex with human IgG1, the authors of this study provide fascinating insights into the antibody-FcR interaction on an atomic level; see also reference 30.
32. Kato, K., Fridman, W. H., Arata, Y. & Sautes-Fridman, C. A conformational change in the Fc precludes the binding of two Fcγ receptor molecules to one IgG. *Immunol. Today* **21**, 310–312 (2000).
33. Zhang, Y. *et al.* Crystal structure of the extracellular domain of a human FcγRIII. *Immunity* **13**, 387–395 (2000).
34. Kinet, J. P. The high-affinity IgE receptor (FcεRI): from physiology to pathology. *Annu. Rev. Immunol.* **17**, 931–972 (1999).
35. Ghazizadeh, S., Bolen, J. B. & Fleit, H. B. Physical and functional association of Src-related protein tyrosine kinases with FcγRII in monocytic THP-1 cells. *J. Biol. Chem.* **269**, 8878–8884 (1994).
36. Wang, A. V., Scholl, P. R. & Geha, R. S. Physical and functional association of the high affinity immunoglobulin G receptor (FcγRI) with the kinases Hck and Lyn. *J. Exp. Med.* **180**, 1165–1170 (1994).
37. Kiener, P. A. *et al.* Cross-linking of Fcγ receptor I (FcγRI) and receptor II (FcγRII) on monocytic cells activates a signal transduction pathway common to both Fc receptors that involves the stimulation of p72 Syk protein tyrosine kinase. *J. Biol. Chem.* **268**, 24442–24448 (1993).
38. Odin, J. A., Edberg, J. C., Painter, C. J., Kimberly, R. P. & Unkeless, J. C. Regulation of phagocytosis and $[Ca^{2+}]i$ flux by distinct regions of an Fc receptor. *Science* **254**, 1785–1788 (1991).
39. Rankin, B. M., Yocum, S. A., Mittler, R. S. & Kiener, P. A. Stimulation of tyrosine phosphorylation and calcium mobilization by Fcγ receptor cross-linking. Regulation by the phosphotyrosine phosphatase CD45. *J. Immunol.* **150**, 605–616 (1993).
40. Liao, F., Shin, H. S. & Rhee, S. G. Tyrosine phosphorylation of phospholipase C-γ 1 induced by cross-linking of the high-affinity or low-affinity Fc receptor for IgG in U937 cells. *Proc. Natl Acad. Sci. USA* **89**, 3659–3663 (1992).
41. Takai, T., Li, M., Sylvestre, D., Clynes, R. & Ravetch, J. V. FcRγ chain deletion results in pleiotrophic effector cell defects. *Cell* **76**, 519–529 (1994).
By generating the γ-chain knockout mouse these authors show the central importance of this molecule for the function of activating FcRs.
42. Clynes, R. & Ravetch, J. V. Cytotoxic antibodies trigger inflammation through Fc receptors. *Immunity* **3**, 21–26 (1995).
43. Park, S. Y. *et al.* Resistance of Fc receptor-deficient mice to fatal glomerulonephritis. *J. Clin. Invest.* **102**, 1229–1238 (1998).
44. Sylvestre, D. L. & Ravetch, J. V. Fc receptors initiate the Arthus reaction: redefining the inflammatory cascade. *Science* **265**, 1095–1098 (1994).
45. Zhang, M. *et al.* Activating Fc receptors are required for antitumor efficacy of the antibodies directed toward CD25 in a murine model of adult T-cell leukemia. *Cancer Res.* **64**, 5825–5829 (2004).
46. Azeredo da Silveira, S. *et al.* Complement activation selectively potentiates the pathogenicity of the IgG2b and IgG3 isotypes of a high affinity anti-erythrocyte autoantibody. *J. Exp. Med.* **195**, 665–672 (2002).
47. Kaneko, Y., Nimmerjahn, F., Madaio, M. P. & Ravetch, J. V. Pathology and protection in nephrotoxic nephritis is determined by selective engagement of specific Fc receptors. *J. Exp. Med.* **203**, 789–797 (2006).
48. Nimmerjahn, F. & Ravetch, J. V. The antiinflammatory activity of IgG: the intravenous IgG paradox. *J. Exp. Med.* **204**, 11–15 (2007).
49. Ravetch, J. V. & Clynes, R. A. Divergent roles for Fc receptors and complement *in vivo*. *Annu. Rev. Immunol.* **16**, 421–432 (1998).
50. Sylvestre, D. *et al.* Immunoglobulin G-mediated inflammatory responses develop normally in complement-deficient mice. *J. Exp. Med.* **184**, 2385–2392 (1996).
51. Uchida, J. *et al.* The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor-dependent mechanisms during anti-CD20 antibody immunotherapy. *J. Exp. Med.* **199**, 1659–1669 (2004).

52. Kumar, V. *et al.* Cell-derived anaphylatoxins as key mediators of antibody-dependent type II autoimmunity in mice. *J. Clin. Invest.* **116**, 512–520 (2006).
53. Schmidt, R. E. & Gessner, J. E. Fc receptors and their interaction with complement in autoimmunity. *Immunol. Lett.* **100**, 56–67 (2005).
54. Barnes, N. *et al.* FcγRI-deficient mice show multiple alterations to inflammatory and immune responses. *Immunity* **16**, 379–389 (2002).
55. Bevaart, L. *et al.* Direct targeting of genetically modified tumour cells to FcγRI triggers potent tumour cytotoxicity. *Br. J. Haematol.* **132**, 317–325 (2006).
56. Fossati-Jimack, L. *et al.* Markedly different pathogenicity of four immunoglobulin G isotype-switch variants of an antierythrocyte autoantibody is based on their capacity to interact *in vivo* with the low-affinity Fcγ receptor III. *J. Exp. Med.* **191**, 1293–1302 (2000).
57. Ioan-Facsinay, A. *et al.* FcγRI (CD64) contributes substantially to severity of arthritis, hypersensitivity responses, and protection from bacterial infection. *Immunity* **16**, 391–402 (2002).
58. Hazenbos, W. L. *et al.* Impaired IgG-dependent anaphylaxis and Arthus reaction in FcγRIII (CD16) deficient mice. *Immunity* **5**, 181–188 (1996).
59. Ji, H. *et al.* Arthritis critically dependent on innate immune system players. *Immunity* **16**, 157–168 (2002).
60. Meyer, D. *et al.* FcγRIII (CD16)-deficient mice show IgG isotype-dependent protection to experimental autoimmune hemolytic anemia. *Blood* **92**, 3997–4002 (1998).
61. Cartron, G. *et al.* Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcγRIIIa gene. *Blood* **99**, 754–758 (2002).
62. Weng, W. K., Czerwinski, D., Timmerman, J., Hsu, F. J. & Levy, R. Clinical outcome of lymphoma patients after idiotype vaccination is correlated with humoral immune response and immunoglobulin G Fc receptor genotype. *J. Clin. Oncol.* **22**, 4717–4724 (2004).
63. Weng, W. K. & Levy, R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. *J. Clin. Oncol.* **21**, 3940–3947 (2003).
References 61–63 demonstrate the importance of FcγRs as effector molecules for antibody therapy of cancer in humans.
64. Bussel, J. B. Fc receptor blockade and immune thrombocytopenic purpura. *Semin. Hematol.* **37**, 261–266 (2000).
65. Stuart, L. M. & Ezekowitz, R. A. Phagocytosis: elegant complexity. *Immunity* **22**, 539–550 (2005).
66. Swanson, J. A. & Hoppe, A. D. The coordination of signaling during Fc receptor-mediated phagocytosis. *J. Leukoc. Biol.* **76**, 1093–1103 (2004).
67. Regnault, A. *et al.* Fcγ receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I-restricted antigen presentation after immune complex internalization. *J. Exp. Med.* **189**, 371–380 (1999).
68. Goodnow, C. C., Sprent, J., de St Groth, B. F. & Vinuesa, C. G. Cellular and genetic mechanisms of self tolerance and autoimmunity. *Nature* **435**, 590–597 (2005).
69. Amigorena, S. *et al.* Cytoplasmic domain heterogeneity and functions of IgG Fc receptors in B lymphocytes. *Science* **256**, 1808–1812 (1992).
70. Muta, T. *et al.* A 13-amino-acid motif in the cytoplasmic domain of FcγRIIB modulates B-cell receptor signalling. *Nature* **369**, 340 (1994).
This study, together with reference 69, was the first to identify and characterize the function of the ITIMs in FcγRIIB.
71. Nitschke, L. & Tsubata, T. Molecular interactions regulate BCR signal inhibition by CD22 and CD72. *Trends Immunol.* **25**, 543–550 (2004).
72. Ono, M., Bolland, S., Tempst, P. & Ravetch, J. V. Role of the inositol phosphatase SHIP in negative regulation of the immune system by the receptor FcγRIIB. *Nature* **383**, 263–266 (1996).
73. Ono, M. *et al.* Deletion of SHIP or SHP-1 reveals two distinct pathways for inhibitory signalling. *Cell* **90**, 293–301 (1997).
74. Pearse, R. N. *et al.* SHIP recruitment attenuates FcγRIIB-induced B cell apoptosis. *Immunity* **10**, 753–760 (1999).
75. Tzeng, S. J., Bolland, S., Inabe, K., Kurosaki, T. & Pierce, S. K. The B cell inhibitory Fc receptor triggers apoptosis by a novel c-Abl-family kinase dependent pathway. *J. Biol. Chem.* **22**, 22 (2005).
76. Radbruch, A. *et al.* Competence and competition: the challenge of becoming a long-lived plasma cell. *Nature Rev. Immunol.* **6**, 741–750 (2006).
77. Odendahl, M. *et al.* Generation of migratory antigen-specific plasma blasts and mobilization of resident plasma cells in a secondary immune response. *Blood* **105**, 1614–1621 (2005).
78. Ravetch, J. V. & Nussenzweig, M. Killing some to make way for others. *Nature Immunol.* **8**, 337–339 (2007).
79. Xiang, Z. *et al.* FcγRIIb controls bone marrow plasma cell persistence and apoptosis. *Nature Immunol.* **8**, 419–429 (2007).
The authors demonstrate that plasma cells are susceptible to FcγRIIB-triggered apoptosis; by contrast, plasma cells from autoimmune mice were more resistant, which might explain their higher abundance in these strains.
80. Dudziak, D. *et al.* Differential antigen processing by dendritic cell subsets *in vivo*. *Science* **315**, 107–111 (2007).
81. Hawiger, D. *et al.* Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions *in vivo*. *J. Exp. Med.* **194**, 769–779 (2001).
This is one of the first studies demonstrating that DCs can functionally inactivate antigen-specific T cells under steady state conditions *in vivo*.
82. Hawiger, D., Masilamani, R. F., Bettelli, E., Kuchroo, V. K. & Nussenzweig, M. C. Immunological unresponsiveness characterized by increased expression of CD5 on peripheral T cells induced by dendritic cells *in vivo*. *Immunity* **20**, 695–705 (2004).
83. Kretschmer, K. *et al.* Inducing and expanding regulatory T cell populations by foreign antigen. *Nature Immunol.* **6**, 1219–1227 (2005).
84. Steinman, R. M. *et al.* Dendritic cell function *in vivo* during the steady state: a role in peripheral tolerance. *Ann. NY Acad. Sci.* **987**, 15–25 (2003).
85. Schuurhuis, D. H., Fu, N., Ossendorp, F. & Melief, C. J. Ins and outs of dendritic cells. *Int. Arch. Allergy Immunol.* **140**, 53–72 (2006).
86. Woelbing, F. *et al.* Uptake of *Leishmania major* by dendritic cells is mediated by Fcγ receptors and facilitates acquisition of protective immunity. *J. Exp. Med.* **203**, 177–188 (2006).
87. Dhodapkar, K. M., Krasovsky, J., Williamson, B. & Dhodapkar, M. V. Antitumor monoclonal antibodies enhance cross-presentation of cellular antigens and the generation of myeloma-specific killer T cells by dendritic cells. *J. Exp. Med.* **195**, 125–133 (2002).
88. Groh, V. *et al.* Efficient cross-priming of tumor antigen-specific T cells by dendritic cells sensitized with diverse anti-MICA opsonized tumor cells. *Proc. Natl Acad. Sci. USA* **102**, 6461–6466 (2005).
89. Rafiq, K., Bergtold, A. & Clynes, R. Immune complex-mediated antigen presentation induces tumor immunity. *J. Clin. Invest.* **110**, 71–79 (2002).
90. Schuurhuis, D. H. *et al.* Immune complex-loaded dendritic cells are superior to soluble immune complexes as antitumor vaccine. *J. Immunol.* **176**, 4573–4580 (2006).
91. Bergtold, A., Desai, D. D., Gavhane, A. & Clynes, R. Cell surface recycling of internalized antigen permits dendritic cell priming of B cells. *Immunity* **23**, 503–514 (2005).
92. Kalergis, A. M. & Ravetch, J. V. Inducing tumor immunity through the selective engagement of activating Fcγ receptors on dendritic cells. *J. Exp. Med.* **195**, 1653–1659 (2002).
93. Boruchov, A. M. *et al.* Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. *J. Clin. Invest.* **115**, 2914–2923 (2005).
94. Dhodapkar, K. M. *et al.* Selective blockade of inhibitory Fcγ receptor enables human dendritic cell maturation with IL-12p70 production and immunity to antibody-coated tumor cells. *Proc. Natl Acad. Sci. USA* **102**, 2910–2915 (2005).
References 92–94 demonstrate the important role of FcγRIIB as a regulator of DC activity and its potential to modulate adaptive immune responses.
95. Nimmerjahn, F. & Ravetch, J. V. Antibodies, Fc receptors and cancer. *Curr. Opin. Immunol.* **19**, 239–245 (2007).
96. Lazar, G. A. *et al.* Engineered antibody Fc variants with enhanced effector function. *Proc. Natl Acad. Sci. USA* **103**, 4005–4010 (2006).
97. Shields, R. L. *et al.* High resolution mapping of the binding site on human IgG1 for FcγRI, FcγRII, FcγRIII, and FcRn and design of IgG1 variants with improved binding to the FcγR. *J. Biol. Chem.* **276**, 6591–6604 (2001).
98. Clynes, R. *et al.* Modulation of immune complex-induced inflammation *in vivo* by the coordinate expression of activation and inhibitory Fc receptors. *J. Exp. Med.* **189**, 179–185 (1999).
99. Yuasa, T. *et al.* Deletion of fcγ receptor IIB renders H-2^b mice susceptible to collagen-induced arthritis. *J. Exp. Med.* **189**, 187–194 (1999).
100. Bergtold, A., Gavhane, A., D'Agati, V., Madaio, M. & Clynes, R. FcR-bearing myeloid cells are responsible for triggering murine lupus nephritis. *J. Immunol.* **177**, 7287–7295 (2006).
101. Arnold, J. N., Wormald, M. R., Sim, R. B., Rudd, P. M. & Dwek, R. A. The impact of glycosylation on the biological function and structure of human immunoglobulins. *Annu. Rev. Immunol.* **25**, 21–50 (2007).
102. Ferrara, C., Stuart, F., Sondermann, P., Brunker, P. & Umana, P. The carbohydrate at FcγRIIIa Asn-162. An element required for high affinity binding to non-fucosylated IgG glycoforms. *J. Biol. Chem.* **281**, 5032–5036 (2006).
This study suggests that the differential impact of fucose on binding to different Fcγ-receptors is dependent on FcγR glycosylation.
103. Shields, R. L. *et al.* Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human FcγRIII and antibody-dependent cellular toxicity. *J. Biol. Chem.* **277**, 26733–26740 (2002).
104. Shinkawa, T. *et al.* The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. *J. Biol. Chem.* **278**, 3466–3473 (2003).
105. Arnold, J. N., Dwek, R. A., Rudd, P. M. & Sim, R. B. Mannan binding lectin and its interaction with immunoglobulins in health and in disease. *Immunol. Lett.* **106**, 103–110 (2006).
106. Bond, A., Cooke, A. & Hay, F. C. Glycosylation of IgG, immune complexes and IgG subclasses in the MRL-lpr/lpr mouse model of rheumatoid arthritis. *Eur. J. Immunol.* **20**, 2229–2233 (1990).
107. Mizuochi, T., Hamako, J., Nose, M. & Titani, K. Structural changes in the oligosaccharide chains of IgG in autoimmune MRL/Mp-lpr/lpr mice. *J. Immunol.* **145**, 1794–1798 (1990).
108. Malhotra, R. *et al.* Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. *Nature Med.* **1**, 237–243 (1995).
109. Nimmerjahn, F., Anthony, R. M. & Ravetch, J. V. Agalactosylated IgG antibodies depend on cellular Fc receptors for *in vivo* activity. *Proc. Natl Acad. Sci. USA* **104**, 8433–8437 (2007).
110. Kaneko, Y., Nimmerjahn, F. & Ravetch, J. V. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* **313**, 670–673 (2006).
The authors demonstrate that the sialic-acid-rich fraction of IVIG is responsible for its anti-inflammatory activity.
111. Bruhns, P., Samuelsson, A., Pollard, J. W. & Ravetch, J. V. Colony-stimulating factor-1-dependent macrophages are responsible for IVIG protection in antibody-induced autoimmune disease. *Immunity* **18**, 573–581 (2003).
112. Debre, M. *et al.* Infusion of Fcγ fragments for treatment of children with acute immune thrombocytopenic purpura. *Lancet* **342**, 945–949 (1993).
This was the first study to show that the IgG Fc-fragment is responsible for mediating the anti-inflammatory activity of IVIG in human patients.
113. Samuelsson, A., Towers, T. L. & Ravetch, J. V. Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science* **291**, 484–486 (2001).
114. Park-Min, K. H. *et al.* FcγRIII-dependent inhibition of interferon-γ responses mediates suppressive effects of intravenous immune globulin. *Immunity* **26**, 67–78 (2007).
115. Siragam, V. *et al.* Intravenous immunoglobulin ameliorates ITP via activating Fc γ receptors on dendritic cells. *Nature Med.* **12**, 688–692 (2006).
116. McGaha, T. L., Sorrentino, B. & Ravetch, J. V. Restoration of tolerance in lupus by targeted inhibitory receptor expression. *Science* **307**, 590–593 (2005).
This study demonstrates the therapeutic potential of increasing FcγRIIB expression on B cells to restore tolerance in autoimmune-prone mouse strains.

117. Vitetta, E. S. & Ghetie, V. F. Immunology. Considering therapeutic antibodies. *Science* **313**, 308–309 (2006).
118. Li, M., Wirthmueller, U. & Ravetch, J. V. Reconstitution of human FcγRIII cell type specificity in transgenic mice. *J. Exp. Med.* **183**, 1259–1263 (1996).
119. Bolland, S. & Ravetch, J. V. Spontaneous autoimmune disease in FcγRIIB-deficient mice results from strain-specific epistasis. *Immunity* **13**, 277–285 (2000).
120. Bolland, S., Yim, Y. S., Tus, K., Wakeland, E. K. & Ravetch, J. V. Genetic modifiers of systemic lupus erythematosus in FcγRIIB$^{-/-}$ mice. *J. Exp. Med.* **195**, 1167–1174 (2002).
121. Fukuyama, H., Nimmerjahn, F. & Ravetch, J. V. The inhibitory Fcγ receptor modulates autoimmunity by limiting the accumulation of immunoglobulin G^{+} anti-DNA plasma cells. *Nature Immunol.* **6**, 99–106 (2005).
122. Takai, T., Ono, M., Hikida, M., Ohmori, H. & Ravetch, J. V. Augmented humoral and anaphylactic responses in FcγRII-deficient mice. *Nature* **379**, 346–349 (1996).
This study describes the generation of the *Fcgriib*-knockout mouse, which provided the first clear evidence of the importance of this protein in regulating immune responses.
123. Paul, E., Nelde, A., Verschoor, A. & Carroll, M. C. Follicular exclusion of autoreactive B cells requires FcγRIIb. *Int. Immunol.* **19**, 365–373 (2007).
124. Nakamura, A. *et al.* Fcγ receptor IIB-deficient mice develop Goodpasture's syndrome upon immunization with type IV collagen: a novel murine model for autoimmune glomerular basement membrane disease. *J. Exp. Med.* **191**, 899–906 (2000).
125. Jiang, Y. *et al.* Polymorphisms in IgG Fc receptor IIB regulatory regions associated with autoimmune susceptibility. *Immunogenetics* **51**, 429–435 (2000).
126. Jiang, Y. *et al.* Genetically determined aberrant down-regulation of FcγRIIB1 in germinal center B cells associated with hyper-IgG and IgG autoantibodies in murine systemic lupus erythematosus. *Int. Immunol.* **11**, 1685–1691 (1999).
127. Pritchard, N. R. *et al.* Autoimmune-prone mice share a promoter haplotype associated with reduced expression and function of the Fc receptor FcγRII. *Curr. Biol.* **10**, 227–230 (2000).
128. Xiu, Y. *et al.* Transcriptional regulation of *Fcgr2b* gene by polymorphic promoter region and its contribution to humoral immune responses. *J. Immunol.* **169**, 4340–4346 (2002).
129. Holmes, M. C. & Burnet, F. M. The natural history of autoimmune disease in Nzb mice. A comparison with the pattern of human autoimmune manifestations. *Ann. Intern. Med.* **59**, 265–276 (1963).
130. Hoyer, B. F. *et al.* Short-lived plasmablasts and long-lived plasma cells contribute to chronic humoral autoimmunity in NZB/W mice. *J. Exp. Med.* **199**, 1577–1584 (2004).
131. Blank, M. C. *et al.* Decreased transcription of the human *FCGR2B* gene mediated by the −343 G/C promoter polymorphism and association with systemic lupus erythematosus. *Hum. Genet.* **117**, 220–227 (2005).
132. Su, K. *et al.* A promoter haplotype of the immunoreceptor tyrosine-based inhibitory motif-bearing FcγRIIb alters receptor expression and associates with autoimmunity. II. Differential binding of GATA4 and Yin-Yang1 transcription factors and correlated receptor expression and function. *J. Immunol.* **172**, 7192–7199 (2004).
133. Su, K. *et al.* A promoter haplotype of the immunoreceptor tyrosine-based inhibitory motif-bearing FcγRIIb alters receptor expression and associates with autoimmunity. I. Regulatory FCGR2B polymorphisms and their association with systemic lupus erythematosus. *J. Immunol.* **172**, 7186–7191 (2004).
134. Floto, R. A. *et al.* Loss of function of a lupus-associated FcγRIIb polymorphism through exclusion from lipid rafts. *Nature Med.* **11**, 1056–1058 (2005).
135. Kono, H. *et al.* FcγRIIB Ile232Thr transmembrane polymorphism associated with human systemic lupus erythematosus decreases affinity to lipid rafts and attenuates inhibitory effects on B cell receptor signaling. *Hum. Mol. Genet.* **14**, 2881–2892 (2005).
In references 134 and 135, the authors elegantly demonstrate that the FcγRIIB I232T allele which is associated with systemic lupus erythematosus in humans is functionally impaired owing to its inefficient association with lipid rafts.
136. Mackay, M. *et al.* Selective dysregulation of the FcγIIB receptor on memory B cells in SLE. *J. Exp. Med.* **203**, 2157–2164 (2006).

Acknowledgements
We would especially like to thank P. Sondermann (Roche), who generously provided the structural data and pictures concerning the antibody FcR interaction and for his critical reading of the manuscript. Similarly, we thank. S. Bolland for suggestions on the manuscript. This work was supported by grants from the German Research Foundation (DFG) and the Bavarian Genome Network (BayGene) to F. N., and by grants from the National Institutes of Health (NIH), USA, and E. Ludwig to J.V.R. We apologize to all colleagues whose important work was not directly cited due to space limitations. These references can be found in the numerous review articles referred to in this Review.

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
Fcgria | *Fcgrib* | *Fcgric* | *Fcgriia* | *Fcgriic* | FcγRIIIA | FcγRIIIB | FcγRIV

FURTHER INFORMATION

Falk Nimmerjahn's homepage:
http://www.molmed.uni-erlangen.de/

ALL LINKS ARE ACTIVE IN THE ONLINE PDF