**New revelations from an old receptor: Immunoregulatory functions of the inhibitory Fc gamma receptor, FcγRIIB (CD32B)**

Ali Roghanian†, ‡\*, Richard J. Stopforth†, Lekh N. Dahal† and Mark S. Cragg†

†Antibody and Vaccine Group, Centre for Cancer Immunology, Cancer Sciences Unit, Faculty of Medicine, University of Southampton, Southampton General Hospital, Southampton, SO16 6YD, UK; ‡Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

**Summary sentence: *Review of the canonical and non-canonical functions of FcγRIIB and its potential as a therapeutic target for immunotherapy of cancer and autoimmune disorders.***

**\*Corresponding author:** Ali Roghanian, Ph.D. Email: aroghani@mit.edu / A.Roghanian@soton.ac.uk

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**Abstract**

The Fc gamma receptor IIB (FcγRIIB/CD32B), was generated million years ago during evolution. It is the sole inhibitory receptor for immunoglobulin (Ig) G, and has long been associated with the regulation of humoral immunity and innate immune homeostasis. However, new and surprising functions of FcγRIIB are emerging. In particular, FcγRIIB has been shown to perform unexpected activatory roles in both immune-signaling and monoclonal antibody (mAb) immunotherapy. Furthermore, although immunoreceptor tyrosine-based inhibitory motif ([ITIM](http://link.springer.com/search?dc.title=ITIM&facet-content-type=ReferenceWorkEntry&sortOrder=relevance)) signaling is an integral part of FcγRIIB regulatory activity, it is now clear that inhibition/activation of immune responses can occur independently of the ITIM. In light of these new findings, we present an overview of the established and non-canonical functions of FcγRIIB and discuss how this knowledge might be exploited therapeutically.

**Abbreviations**

Antigen: Ag

B cell receptor: BCR

Bruton’s tyrosine kinase: Btk

Collagen induced arthritis: CIA

C-reactive protein: CRP

Demyelinating polyneuropathy: CIDP

Dendritic cell: DC

Experimental autoimmune encephalomyelitis: EAE

Fc gamma receptor: FcγR

Hematopoietic stem cells: HSC

Human: h

Idiopathic thrombocytopenic purpura: ITP

Immune complex: IC

Immunoglobulin: Ig

Immunoreceptor tyrosine-based inhibitory motif: [ITIM](http://link.springer.com/search?dc.title=ITIM&facet-content-type=ReferenceWorkEntry&sortOrder=relevance)

Intravenous immunoglobulin: IVIg

Isoleucine: Ile

Immunoreceptor tyrosine-based activation motif: ITAM

Liver sinusoidal endothelial cells: LSEC

Monoclonal antibody: mAb

Plasmacytoid DCs: pDC

Phosphatidyl inositol-3,4-bisphosphate: PIP2

Phosphatidyl inositol 3’ kinase: PI3K

SH2-domain phosphatase-1 (SHP-1)

Src homology-2 domain-containing inositol 5-phosphatase 1: SHIP-1

Systemic lupus erythematosus: SLE

RAS-related protein Rab family: RAB

Rheumatoid arthritis: RA

Threonine: Thr

TNF receptor: TNFR

**Introduction**

There are six FcγRs in humans, four in mice, but only a single inhibitory receptor in each species; FcγRIIB (CD32B) [1, 2]. FcγRIIB inhibits and regulates various immune cells through its effects on the activatory FcγRs, as well as other activatory receptors, and for almost 2 decades it has been known to impair certain types of mAb therapy. Over the past decade however, new and surprising functions of FcγRIIB have been revealed, and we have witnessed considerable progress in the understanding of its multiple functions. FcγRIIB has been shown to elicit a hitherto unrecognized means of resistance to clinically important hematological mAbs such as rituximab (anti-CD20) and alemtuzumab (anti-CD52) [3-5]. Contrary to accepted dogma, new studies have also shown its pivotal role in enhancing immune activation by agonistic antibodies to TNF receptor (TNFR) superfamily members, such as CD40 [6-8], as well as providing an explanation for the unexpected cytokine release seen with the CD28 agonist mAb TGN1412 [9, 10]. Reagents capable of binding and manipulating FcγRIIB are now available [11-13], providing the potential to fine-tune these immunotherapy strategies. Furthermore, although inhibition mediated via the cytoplasmic ITIM domain was considered to be a cardinal feature of FcγRIIB-mediated immune regulation, it is now clear that inhibition/activation of immune responses can in certain cases occur independently of ITIM signaling. Here, we discuss these novel facets of FcγRIIB biology as relevant to aspects of immune regulation ranging from therapeutic mAbs and homeostatic immunity to disease prevalence and severity; highlighting how targeting FcγRIIB may be therapeutically exploited.

**FcγRIIB structure**

FcγRIIB is a 40 kDa glycoprotein consisting of two Ig-like extracellular domains, a transmembrane region and a cytoplasmic tail containing an [ITIM](http://link.springer.com/search?dc.title=ITIM&facet-content-type=ReferenceWorkEntry&sortOrder=relevance). In humans and mice, alternative splicing of FcγRIIB transcripts results in FcγRIIB1 and FcγRIIB2 isoforms. FcγRIIB1 has a longer cytoplasmic tail (19 and 47 amino acids in humans and mice, respectively) which stops it from entering coated pits and prevents endocytosis/internalization of the receptor following engagement of immunoglobulin (Ig) G immune complexes (IC) [14, 15](Figure 1). These were demonstrated in a number of seminal papers in the early 1990’s, where cell lines were transfected with the two isoforms and their differences in IC binding and regulation of B cell receptor (BCR) activation investigated in detail [14-16]. Our group further demonstrated this in the context of therapeutic mAb internalization upon binding to B cell surface antigens (Ag), where mAb was mainly internalized in the presence of the human FcγRIIB2 isoform [17]. FcγRIIB1 is predominantly expressed on B cells, whereas, myeloid cells express predominantly the B2 isoform [18, 19].

**FcγRIIB signaling**

FcγRIIB represents a prototypic example of an inhibitory receptor, reducing downstream signaling of activatory receptors, provided that it is both co-expressed on the same cell, and co-localizes, with the activatory receptor following ligand-induced crosslinking (Figure 2). In B cells, colocalization of the BCR with FcγRIIB1 by Ag-antibody ICs limits B cell expansion[20]. It actively downregulates B cell activation by increasing the threshold for BCR activation and suppresses B cell-mediated Ag presentation to T cells [21]. In the absence of BCR ligation, FcγRIIB1 signaling can also induce mature B cell apoptosis [22]. Therefore, FcγRIIB1 provides three mechanisms pivotal to maintenance of B cell tolerance - apoptosis of self-reactive B cells, follicular exclusion of low-affinity autoreactive B cells and curtailing B cell activation.

The ITIM domain (and specifically a single tyrosine residue, phosphorylated following stimulation) in the cytoplasmic domain of FcγRIIB1, is responsible for inhibition of Ca2+ flux downstream of BCR stimulation through the activation of phosphatases.To determine the dominant phosphatase mediating FcγRIIB1’s inhibitory effects, Ono et al. generated a range of FcγRIIB chimeric receptors and cell lines deficient in Src homology-2 domain-containing inositol 5-phosphatase 1 (SHIP-1) or SH2-domain phosphatase-1 (SHP-1) [23] and confirmed a dominant role for SHIP-1[23]. Through its 5’ inositol phosphatase activity [24], SHIP-1 converts membrane localized phosphatidyl inositol-3,4,5-triphosphate (PIP3), itself generated by phosphatidyl inositol 3’ kinase (PI3K), to phosphatidyl inositol-3,4-bisphosphate (PIP2). This consequently prevents recruitment of Pleckstrin homology domain-containing signaling proteins such as Bruton’s tyrosine kinase (Btk) to the cell membrane (thereby limiting activation of pathways that result in Ca2+ flux/activation) [25]. This may also favor recruitment of proteins such as TAPP1/2 that preferentially bind to PIP2 [26]. As recently reviewed elsewhere [27], further to its phosphatase activity, SHIP-1 may also modulate Ras pathway activation by interacting with molecules such as Shc and Dok-1.

FcγRIIB2 can similarly inhibit signaling from activatory receptors co-expressed on myeloid cells (Figure 2). Amongst others, this has been shown for FcγRIIA (on basophils/monocytes/macrophages) and the IgE receptor FcεRI (on mast cells/basophils) [28]. Studies in the THP-1 monocytic cell line indicate that crosslinking of FcγRIIB2 induced SHIP-1 phosphorylation and decreased Akt phosphorylation in comparison to FcγRIIA crosslinking alone [29]. For the IgE receptor, FcγRIIB was phosphorylated following crosslinking with FcεRI, which recruited and activated SHIP-1[30]. As FcγRIIB2 is capable of inhibiting serotonin release downstream of FcεRI stimulation in SHP-1-deficient mast cells [31], this supports an important role for SHIP-1.

FcγRIIB2 expression on myeloid cells is of importance for several reasons. Firstly, in contrast to the context of the BCR, FcγRIIB2 will colocalize with activatory FcγRs including FcγRIIA following binding to ICs, in an Ag non-specific manner. Secondly, the efficacy of mAb therapies, which require activatory FcγRs for their functions, is limited by FcγRIIB2 interaction [32] (see below).

In addition to these established canonical signaling functions of FcγRIIB, a recent report has provided a new capability, defined as *trans* inhibition [33]. Whereas *cis*-inhibition represents the inhibition (by FcγRIIB) of signaling downstream of physically associated activatory receptors (Figure 2A), *trans* inhibition involves inhibition of signaling from a distinct activatory receptor complex that is not associated with FcγRIIB and may have a distinct ligand (Figure 2B). The authors also highlighted a fundamental role for SHIP-1 in this mechanism [33].

## *FcγRIIB effects independent of the cytoplasmic domain*

Although many of the canonical functions of FcγRIIB require signaling through the cytoplasmic ITIM as detailed above, in certain cases the modulation of activity is achieved independently of the cytoplasmic domain. For example, in mouse IIA1.6 B lymphoma cells, a cytoplasmic domain-truncated variant of FcγRIIB was shown to retain the ability to inhibit phosphorylation of CD19 following crosslinking with the BCR [34]. Similarly, using high-resolution, high-speed, live imaging approaches, Xu and colleagues [35] recently demonstrated that when co-ligated to the BCR, FcγRIIB1 can inhibit B cell activation by blocking the colocalization of BCR and CD19 microclusters within the B cell immunological synapse, in an ITIM-independent manner requiring the transmembrane domain (Figure 2C). Further, they showed that primary human B cells from SLE patients homozygous for the FcγRIIB 232 threonine (Thr) polymorphism which results in defective signaling (see below) failed to block the synaptic colocalization of the BCR with CD19, leading to dysregulated recruitment of activated PI3K to the membrane proximal signalosome [35].

***FcγRIIB-mediated crosslinking of therapeutic antibodies***

Recent studies investigating mechanisms underlying the efficacy and function of various therapeutic mAbs have also shed new light into novel functions of FcγRIIB (reviewed in [7, 19]). On normal and malignant B cells, FcγRIIB has been shown to be involved in removing the clinically relevant CD20 mAb, rituximab, from the B cell surface, effectively abrogating its cell-mediated anti-cancer mechanisms [3]. This FcγRIIB-mediated internalization is independent of the ITIM, as cells transfected with FcγRIIB-ITIM mutants and intracellular domain-null FcγRIIB show equal internalization to cells expressing the WT receptor [4, 17]. We recently investigated this effect of FcγRIIB on other cell surface receptors (CD19, CD22, CD37, CD38, CD40, MHCII, and the IgM BCR) and demonstrated that when targeted by a mAb, certain other Ags are also modulated in a similar manner, albeit not to the extent of rituximab and other type I CD20 mAbs [4, 36] (Figure 3A).

Data generated by independent groups also recently provided surprising evidence that FcγRIIB acts to potentiate the immunostimulatory activities of certain therapeutic mAbs in vitro and in vivo [6, 8, 37]. In particular, this property has been shown for mAbs targeting TNFR family members such as CD40, OX40, 4-1BB and GITR with isotypes such as mIgG1, which have a high affinity for FcγRIIB, providing maximal immunostimulatory activity. Importantly, this activity is independent of the FcγRIIB ITIM and intracellular tail, as cells expressing just the extracellular domain of the receptor provide equivalent immunostimulation to WT receptor both in vitro[6] and in vivo [38] (Figure 3B). Indeed the FcγRIIB-dependency and crosslinking of therapeutic mAbs can be bypassed through mAb multimerization, further excluding the requirement for any active signaling from FcγRIIB [39].

This ability of FcγRIIB to elicit enhanced agonistic function is not always beneficial however. In the Phase 1 trial of a humanized CD28-specific IgG4 mAb (TGN1412), six healthy volunteers experienced a life-threatening, systemic inflammatory response with elevated levels of proinflammatory cytokines leading to severe clinical symptoms [40]. The failings of the pre-clinical safety assays and the trial design overall have been extensively discussed elsewhere [41], but one relevant facet relates to FcγRIIB expression. Although TGN1412 fails to stimulate human PBMCs cultured at normal density (106 cells/ml), PBMCs pre-cultured at high density (107 cells/ml) react vigorously to TGN1412 treatment [42]. FcγRIIB expression is logarithmically increased on monocytes during this high density preculture and studies have shown that FcγRIIB is necessary and sufficient to hyper cross-link TGN412 and deliver potent cytokine secretion [9].  This is interesting and unexpected as, unlike B cells, circulating monocytes express little or no FcγRIIB [9].  In agreement with this, co-incubation of TGN1412-treated T cells with sufficient B cells expressing FcγRIIB, and with FcγRIIB transfectants, results in robust T cell activation [10]. Since B cells represent ~33% of lymphoid tissues, it is expected that they are the main cell type providing equivalent crosslinking of TGN1412 in vivo [43]. However, depending on the context, high FcγRIIB may also be present on neighboring myeloid cells in vivo, as is frequently the case within various microenvironments and under certain stimuli.

**FcγRIIB expression and function on leukocytes and non-leukocytes**

In both human and rodents FcγRIIB is expressed on multiple cell-types, where it can modulate their functions and activation state. As most of these have extensively been reviewed in the past[21], here we highlight those which have been studied in more detail in recent years.

***Regulation of dendritic cell (DC) function***

# DCs co-express both activatory and inhibitory FcγRs, the balance of which helps regulate their activation status. FcγRIIB-mediated ITIM signaling contributes to the maintenance of immature/tolerogenic property of DCs, resulting in the suppression of autoreactive T cells and generation of autoantibodies [44, 45]. In mouse, FcγRIIB contributes to peripheral tolerance maintenance by inhibiting DC activation and/or by limiting processing of exogenously acquired Ag. In this regard, FcγRIIB inhibits CD8+ T cell priming via suppression of both DC activation and cross-presentation through activatory FcγRs [46]. Mouse DCs lacking FcγRIIB have an improved capacity to activate naïve T cells and induce CD8+ T cell expansion in vivo, which is due to their enhanced FcγR-dependent Ag presentation [47]. Moreover, FcγRIIB limits antibody-mediated cross-priming of DCs and FcγRIIB-deficient rat insulin promoter-mOVA mice have increased diabetic sensitivity [48]. Furthermore, FcγRIIB-deficient mice fail to develop mucosal tolerance to OVA as FcγRIIB expression modulates the Ag-presenting capacity of DCs. FcγRIIB-deficient DCs under tolerogenic conditions have increased IgG-induced release of inflammatory cytokines such as MCP-1, TNF-α, and IL-6 as well as higher expression of surface costimulatory molecules, resulting in an altered T cell response associated with increased IL-2 and IFN-γ secretion in vitro [49]. Similarly, FcγRIIB-deficient DCs have a reduced ability to take up ICs and display decreased T cell stimulation in vitro with less efficient IgG production[50]. In a murine asthmatic model, Ag-specific IgG ameliorated the Ag transporting and presenting capacity on CD11c+ APCs (including DCs), attenuating allergic airway inflammation via FcγRIIB by shifting from a Th2 to a Th1 response [51].

# High expression of FcγRIIB and the lack of activatory FcγRs on murine plasmacytoid DCs (pDC) preclude efficient Ag presentation of these cells compared to conventional DCs [52]. In keeping with this, the dominance of FcγRIIB on mouse pDCs acts as a mechanism to prevent IFN-α production by TLR-bearing ICs. The FcγRIIB-specific means of controlling pDC-derived IFN-α may also be important during chronic infections when circulating viral ICs are present [53].

Using intravital two-photon microscopy, it has been demonstrated that dermal DC migration to lymph nodes is also affected by FcγRIIB (being accelerated in its absence). These observations have relevance to autoimmunity, since autoantibody-containing serum samples from humans and mice with SLE also increase dermal DC migration in vivo, potentially driving inappropriate localization of autoAg-bearing DCs [54].

***Regulation of memory CD8+ T cells by FcγRIIB***

The expression of FcγRs by T cells is controversial and has been questioned in the literature [55]. However, microarray analysis has shown that *f*cgr2b mRNA, but not activatory *fcgr* mRNA, is upregulated in memory CD8+ T cells generated after Listeria monocytogenes infection [56], indicating a possible regulatory function for FcγRIIB in controlling IC-mediated cytotoxic T cell responses. More recent evidence also suggests that the majority of memory CD8+ T cells generated by bacterial or viral infections express only FcγRIIB, and that FcγRIIB can be detected on the surface of previously activated human CD8+ T cells [57]. Of note, FcγR stimulation during in vivo Ag challenge not only inhibits the cytotoxicity of memory CD8+ T cells against peptide-loaded or virus-infected targets, but FcγRIIB blockade during homologous virus challenge enhances the secondary CD8+ T cell response [57].

***FcγRIIB expression and function on endothelial cells***

It has long been known that liver sinusoidal endothelial cells (LSEC) express high levels of FcγRIIB, thereby controlling IC half-life as well as IC-mediated autoimmune disease and potentially infection. In rats, FcγRIIB2 has been shown to be the dominant FcγR expressed by LSECs which undergoes extensive recycling upon IC binding. An unexpectedly large proportion of FcγRIIB2 that is recycled back to the cell surface is apparently bound with ICs [58]. Similarly in mice ~75% of FcγRIIB has been reported to be expressed in the liver with most (90%) expressed on LSEC, with relatively little on the Kupffer cells. FcγRIIB-deficient mice infused intravenously with radio-iodinated small ICs made of OVA and rabbit IgG anti-OVA are severely deficient in eliminating ICs compared with WT mice; terminal half-lives being 6 and 1.5 hours, respectively [59]. Collectively therefore, FcγRIIB on LSECs is believed to act as a scavenger, keeping small IC concentrations low in the circulation and minimizing pathologic deposition of inflammatory ICs [58, 59].

The high FcγRIIB expression on LSECs potentially represents an issue in targeting the receptor therapeutically. We observed that murine FcγRIIB mAbs were rapidly and extensively consumed in vivo leading to a significant loss from the circulation [60]. Furthermore, high interaction of ICs or therapeutic mAbs with human (h) FcγRIIB on LSECs may cause unwelcome liver toxicity and side effects. However, our recent observations with human liver and hFcγRIIB Tg mice indicate that hFcγRIIB is not as highly expressed on human LSECs; and hence we do not anticipate similar issues with hFcγRIIB mAbs in man [13, 61].

FcγRIIB (B2) is also expressed within the villus interstitium of the placenta where it may serve as an additional IgG transporter from mother to fetus as well as perhaps an IC scavenging receptor [62-65]. To formally address this hypothesis, Ishikawa and coworkers performed in vitro bioimaging analysis of IgG trafficking using human umbilical vein endothelial cells. They demonstrated that the Rab family of proteins [RAS-related protein Rab family (RABs)], and in particular RAB3D which is predominantly expressed in placental endothelial cells, were associated with FcγRIIB2 compartments and participated in the transcytosis of IgG [65].

FcγRIIB is also expressed in skeletal muscle microvascular endothelium, but not skeletal muscle myocytes, adipocytes or hepatocytes. Here, C-reactive protein (CRP) binding to FcγRIIB causes dephosphorylation of endothelial nitric oxide (NO) synthase and during inflammation CRP activation of FcγRIIB causes impaired insulin-mediated endothelial action, impaired muscle glucose delivery, and insulin resistance [66]. CRP levels are strongly correlated with increased risk of cardiovascular disease. In this regard, Mineo et al. [67] using both gain- and loss-of-function strategies in vitro and in vivo, demonstrated that FcγRIIB underlies the actions of CRP on vascular endothelium. These findings provide novel evidence whereby CRP, and by inference FcγRIIB, as well as circulating ICs, may contribute to the pathogenesis of vascular disease.

These observations over the past few decades indicate that FcγRIIB plays important roles in fine-tuning various cellular/physiological functions within the body and its dysregulation may lead to various disorders. A key question relates to how these effects are mediated; principally through intracellular signaling.

**FcγRIIB dysregulation in autoimmunity**

As discussed above, mechanisms governing central and peripheral B cell tolerance are critically regulated by FcγRIIB, such that autoreactive B cells are not seeded into the periphery. However, failure of these mechanisms leads to activation of autoreactive B cells resulting in autoimmunity. In humans, the best evidence for this comes from studies in which immunecompromised NOD/SCID/Il2rg mice were engrafted with human hematopoietic stem cells (HSC) carrying FcγRIIB-232 isoleucine (Ile) or FcγRIIB-232Thr alleles [68]. These alleles are encoded by non-synonymous thymidine-to-cytosine transitions in exon 5 of the *fcgr2b* gene resulting in the replacement of Thr for Ile at position 232 within the transmembrane region (Figure 1); with the FcγRIIB-232Thr variant associated with systemic lupus erythematosus (SLE) [69-72]. This variant displays a defect in partitioning to lipid rafts [72, 73], reducing its ability to inhibit activatory signaling initiated by activatory FcγRs or the BCR [72]. Humanized mice carrying two copies of the 232Thr allele had a higher level of serum IgM, IgG and autoantibodies, with higher levels of autoantibody-producing plasma cells in the bone marrow compared to Ile/Thr and Ile/Ile donors, firmly establishing an important role for this polymorphism and FcγRIIB itself as a gatekeeper of humoral tolerance in the human immune system in vivo [68]. In some animal models, FcγRIIB deficiency results in similar increase in serum levels of anti-DNA autoantibodies, increased frequency of anti-DNA reactive IgG+ B cells with plasma cell phenotype, IC deposition in the glomeruli and renal disease[74]. Similarly, strains of mice that develop spontaneous autoimmune disease, such as NZB, NOD, BXSB, and MRL/lpr, have been shown to express reduced levels of FcγRIIB on activated or germinal-center B cells [75]. Restoration of FcγRIIB expression by retroviral transduction of bone marrow cells leads to improved survival of experimental animals [75]. In the experimental autoimmune encephalomyelitis (EAE) model, FcγRIIB-deficientmice have a higher EAE severity score and activated Ag-specific effector T cells which, when adoptively transferred to naïve recipients, are able to induce EAE [76]. In the reverse scenario, deficiency of the activatory FcγRIII protects mice from disease and elicits T cells which fail to adoptively transfer EAE [76]. In otherwise resistant strains of mice, FcγRIIB deficiency results in several antibody- or IC-dependent autoimmune symptoms such as alveolitis and lupus nephritis which can be mitigated by transgenic overexpression of hFcγRIIB on B cells [77]. FcγRIIB deficiency is also associated with elevated collagen-specific IgG titres and disease severity in collagen induced arthritis (CIA) models [78]. In humans, similar associations are seen. In rheumatoid arthritis (RA) patients, expression of FcγRIIB is significantly reduced on memory B cells and plasmablasts compared to healthy donors [79]. Immunohistochemical staining of splenic tissues from idiopathic thrombocytopenic purpura (ITP) patients also shows a substantially reduced expression of FcγRIIB on macrophages compared to healthy controls, suggesting that levels of FcγRIIB may serve as a biomarker in ITP [80]. Polymorphisms in the hFcγRIIB gene within both promoter and coding regions have been identified and associated with autoimmunity (reviewed in [81]).

Expression of FcγRIIB on different cell subsets also carries different immunological consequences in autoimmunity. Whilst FcγRIIB is up-regulated on memory B cells in non-autoimmune individuals, it is considerably decreased in memory B cells of SLE patients, which also directly correlates to decreased FcγRIIB-mediated suppression of BCR-induced calcium response [82]. However, deficiency of FcγRIIB on B cells is only a susceptibility factor under the influence of epistatic modifiers for the development of autoimmunity [83, 84]. Thus, the presence of FcγRIIB on B cells would simply be a prerequisite for autoantibody production and may have only a minor effect on disease susceptibility, whereas its presence on myeloid effector cells, as a modulator of the threshold for the downstream antibody effector pathway may play a dominant role in the susceptibility for autoimmune pathology [78]. For example, overexpression of FcγRIIB on B cells seems to have no effect on the frequency or disease course of CIA, but results in early resolution, reduction in joint destruction and reduced levels of autoantibodies, whilst overexpression on macrophages results in reduced bacterial phagocytosis rather than an effect on the autoimmune disease process [77]. These data suggest that prior to developing therapies, it will be important to carefully consider whether modulation of FcγRIIB expression/signaling on B cells and/or cells of myeloid origin is desirable to achieve therapeutic benefit in a given disease setting.

One treatment that is routinely used to treat systemic inflammatory and/or autoimmune diseases such as ITP and SLE, and inflammatory neuropathies such as chronic inflammatory demyelinating polyneuropathy (CIDP) is intravenous infusion of Ig (IVIg).

***Role of FcγRIIB in intravenous immunoglobulin (IVIg) therapy***

How IVIg works is vigorously debated and several competing mechanisms of action cited as central mediators[55], including those dependent upon FcγRIIB. In lupus-prone and healthy animals, IVIg was shown to upregulate the expression of FcγRIIB on B cells with the associated biological consequences [85]. Mechanistically, this may relate to the fact that targeted overexpression of FcγRIIB on B cells can increase the threshold for B cell activation and suppress autoimmune disease[86]. In contrast, untreated CIDP patients consistently express lower levels of FcγRIIB on naïve and memory B cells compared to healthy controls. Whether the therapeutic effect is through B cells is not clear, as following clinically effective IVIg therapy, FcγRIIB protein expression is upregulated on both monocytes and B cells [87]. However, a recent clinical study showed no significant differences in the absolute number or percentage of FcγRIIB+ monocytes in children with ITP (*n* = 20) following IVIg treatment [88] and so a role for B cells may be more likely.

Although the involvement of FcγRIIB in IVIg therapy has been established, and mice lacking FcγRIIB do not benefit from IVIg infusions in models of arthritis or nephritis, whether or not sialylation of the Fc core polysaccharide of the IVIg is required for its anti-inflammatory properties is debated. Studies from the Ravetch lab have indicated that sialylation of the N-linked (Asn297) carbohydrate facilitates DC-SIGN binding on macrophages and DCs to activate FcγRIIB[89, 90]. Other studies have directly contested this and shown that FcγRIIB binding is independent of the Ig sialylation state [91-93]. Nevertheless, it appears clear that the positive outcome following IVIg therapy is associated with a favorable resetting of the activatory:inhibitory FcγR (A:I) ratio [94] and is at least partially mediated by FcγRIIB.

**Regulation of FcγRIIB during infection**

The innate immune system relies on its capacity to rapidly detect invading pathogenic microbes as foreign and to eliminate them. However, pathogens have evolved a myriad of strategies to avoid or bypass the immune system. For example, *Listeria monocytogenes* allows itself to be engulfed by macrophages and then escapes from the phagosome into the cytoplasm where it replicates ([95]). In parallel, the host has also evolved to limit over-active immune responses in order to limit tissue damage; FcγRIIB modulations are an important component of these responses. FcγRIIB-deficient macrophages have increased phagocytosis of Streptococcus pneumoniae in vitro and increased bacterial clearance and survival is seen in infected FcγRIIB-deficient mice. The downside of this hyper-activation is that previously immunized FcγRIIB-deficient mice challenged with large inocula have reduced survival, correlated with increased production of sepsis-associated pro-inflammatory cytokines (e.g., TNF-α and IL-6) [96]. Conversely, although overexpression of FcγRIIB on murine macrophages has little effect on autoimmune disease or on immune responses, it reduces bacterial phagocytosis and survival in response to Streptococcus pneumonia [77]. On the other hand, certain pathogens can directly bind to and activate FcγRIIB on immune effector cells. For instance, binding of glucuronoxylomannan, the major polysaccharide component of Cryptococcus neoformans, to FcγRIIB results in recruitment of SHIP-1 in monocytes and macrophages, with a subsequent inhibition of cytokines (e.g., TNF-α) [97].

Collectively, reduced expression of FcγRIIB results in heightened anti-infective immune responses, but its over-activation can be fatal and hence the presence of FcγRIIB is essential to maintain a response of the appropriate magnitude. Nonetheless, FcγRIIB can be transiently down-regulated to boost acute immune-responses when required, for example during immunization.

**FcγRIIB and immunization**

As expected and given its broad expression on leukocytes, FcγRIIB downregulates immunization responses in vivo by setting the upper threshold for Ab production. As such, FcγRIIB-deficient animals display elevated IgG levels in response to both T-dependent and T-independent Ags [98]. FcγRIIB deficiency results in an increase in IgG antibodies to DNA in the serum, an increased frequency of anti-DNA-reactive IgG+ B cells with a plasma cell phenotype and IC deposition in the glomeruli and renal disease in mice [74].  In agreement with this, in FcγRIIB-deficient mice, mouse IgG2a ICs enhanced primary Ab responses, development of germinal centers and immunological memory [99]. Inhibition of mouse FcγRIIB with an antagonistic mAb (AT128 [12]) has also been shown to suppress recall responses to human FVIII ex vivo, presumably by inhibiting binding of FVIII ICs to FcγRIIB expressed by splenic memory B cells and/or effector cells [100].

**FcγRIIB as a therapeutic target**

It has long been appreciated that FcγRIIB dysregulation and A:I FcγR ratio imbalance have detrimental effects in the host and limit certain immunotherapy treatments, making FcγRIIB an attractive therapeutic target. In the case of cancer therapy, this was originally demonstrated by Clynes et al., who demonstrated mAb therapy was significantly improved in FcγRIIB-deficient mice [32] (Figure 3C). Since this approach is not viable in the clinic, recalibration of the FcγR expression profile and A:I ratio is an attractive way by which to augment mAb function. FcγRIIB can be targeted by specific blocking mAbs or its expression regulated by modulating the tumor microenvironment [11, 13, 101, 102]. Alternatively, engineering therapeutic mAbs to reduce their binding to FcγRIIB has been proposed as an approach to overcome such resistance [19, 103].

As discussed above, we demonstrated that high FcγRIIB expression on the surface of malignant B-cells is a biomarker for therapeutic mAb internalization[3, 4]; reducing mAb half-life and competing with activatory FcγRs on neighboring effector cells(Figure 3A) [102]. We went on to show that FcγRIIB on mantle cell lymphoma and follicular lymphoma cells is a biomarker of poor response to rituximab-therapy [3, 104]. Using state-of-the-art humanized mouse models of lymphoma, the Chen and Hemann labs identified hFcγRIIB as one of the key bone marrow-resistant lymphoma biomarkers in response to alemtuzumab; with FcγRIIB-/- human lymphoma cells sensitized to mAb-therapy in vivo [5]. These findings were recently extended to human SLE and RA patient B cells [105]. Therefore, targeting FcγRIIB with specific blocking mAbs may be a strategy to effectively clear FcγRIIB+ tumor/autoimmune B cells, via (i) opsonizing the target cells; and (ii) inhibiting FcγRIIB-negative regulation of effector cells [13, 102].

Rankin *et al*. showed that targeting FcγRIIB on malignant human B cell-lines by a humanized hFcγRIIB specific mAb (2B6) could be efficacious as a monotherapy [106]. However, this study used xenograft systems, where the target Ag was expressed only on the tumor, and not critical immune effectors, precluding assessment of the net effects of FcγRIIB mAb. To extend these findings we used the n-CoDeR® phage-display library, to generate a series of specific, fully human, hFcγRIIB mAbs [13]. These mAbs were assessed for the ability to selectively bind and block IC binding to hFcγRIIB, but not hFcγRIIA. We demonstrated in immunocompetent syngeneic mouse models where hFcγRIIB is expressed on both the target B cells and effectors, that antagonistic FcγRIIB mAbs have intrinsic target cell depleting activity and can synergize with rituximab and other therapeutic mAb [13]. The clinical candidate BI-1206 is currently in early stage Phase I clinical trials in non-Hodgkin’s lymphoma patients in the UK. We believe the antagonistic hFcγRIIB mAb can promote target cell depletion through at least three independent mechanisms of action; (i) by directly binding to FcγRIIB on targets and inducing programmed-cell death, antibody-dependent cell- cytotoxicity and -phagocytosis, (ii) by preventing interaction of other therapeutic mAb (e.g., rituximab) with FcγRIIB and stabilizing the mAb for longer interaction with effector cells, and finally (iii) by sequestering FcγRIIB away from immune synapses on effector cells and hence allowing efficient interaction of activatory hFcγRs with the therapeutic mAbs [13, 107].

Recently, attempts have been made to generate novel bispecific scaffolds with FcγRIIB for therapeutic applications. Veri and colleagues developed a molecule with an Fv region from 2B6 directed against FcγRIIB coupled to an Fv region from a mAb directed against CD79B [108]. Dual targeting of B cells by FcγRIIB and CD79B inhibited calcium mobilization, cell proliferation, and Ig secretion in vitro and evoked control of CIA in mice [108]. Similarly, FcγRIIB x FcγRIII molecules have potent, dose-dependent cytotoxicity in retargeting human leukocytes against B-lymphoma cell-lines as well as in mediating autologous B cell depletion in culture. Additionally, FcγRIIB x FcγRIII molecules are stable in vivo and effectively delete lymphoma xenografts [109]. These bispecific molecules are currently under development and may soon find their way into human clinical applications.

**Concluding Remarks**

In summary it is clear that there is still much to learn from the study of FcγRs and FcγRIIB in particular. The last decade has shown us remarkable and unexpected new functions for this receptor. Central to immune regulation during immunization, pathology and therapy, the complex roles of FcγRIIB warrant further study. With the recent development of reagents capable of manipulating this key receptor, including a number of specific antagonistic and agonistic FcγRIIB mAbs (both human and mouse), we will surely learn more in the coming decade. Furthermore, therapeutic interventions manipulating hFcγRIIB expression and function in various settings, including in oncology and autoimmunity, may be added to the already growing list of immunomodulatory drugs to be exploited either alone or in combination with other therapies.

**Authorship**

All authors contributed equally to the production of this review.

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**Conflict of Interest Disclosure**

M.S.C. is a retained consultant for BioInvent International and has performed educational and advisory roles for Baxalta. He has received research funding from Roche, Gilead and GSK. A.R. receives funding from BioInvent International.

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**Figure Legends**

**Figure 1. Schematic structures of human and mouse FcγRIIB and their respective polymorphic variants.** The two extracellular Ig-like domains, a transmembrane region (with the 232 Ile to Thr non-synonymous polymorphism in hFcγRIIB) and the cytoplasmic tail containing an [ITIM](http://link.springer.com/search?dc.title=ITIM&facet-content-type=ReferenceWorkEntry&sortOrder=relevance) domain indicated. Alternative splicing of FcγRIIB transcripts results in FcγRIIB1 and FcγRIIB2 isoforms (insertion of 19 and 47 intracellular amino acids in human [ALPGYPECREMGETLPEKP] and mouse [HREMGETLPEEVGEYRQPSGGSVPVSPGPPSGLEPTSSSPYNPPDLE], respectively) also highlighted. Source: UniProt and Protter.

**Figure 2. Mechanisms of inhibition of signaling by FcγRIIB.** (A) FcγRIIB is responsible for the inhibition of signaling (and downstream consequences such as cellular activation) following co-ligation with distinct ITAM-containing activatory receptors, as depicted for the BCR on B cells (left), or activatory FcγR on myeloid cells (right). SHIP-1, the phosphatase required for this inhibitory function of FcγRIIB, is shown to be recruited to the phosphorylated ITIM of FcγRIIB. (B) FcγRIIB-activatory FcγR co-ligation may inhibit signaling downstream of distinct activatory FcγR complexes (‘*trans*-inhibition’), in a SHIP-1-dependent manner. C) FcγRIIB can also limit the co-localization of the BCR with CD19, a key step in BCR signaling. The absence of the FcγRIIB cytoplasmic tail represents the finding that this domain is not required in this setting. P; phosphorylated tyrosine

**Figure 3. Distinct functions of FcγRIIB in therapeutic settings.** (A)FcγRIIB has been shown to participate in the internalization of a number of therapeutic Ab/Ag complexes (e.g., rituximab/CD20) following opsonization of target B cells. This represents a resistance mechanism in the setting of therapeutic depletion of malignant or autoimmune B cells with rituximab. (B) FcγRIIB on an effector cell is shown, contributing to the clustering of anti-TNFR superfamily member mAbs (i.e., anti-DR5/CD40) bound to a target cell in *trans*. This has been shown to promote either pro-apoptotic or agonistic signaling (e.g., NF-κB signaling) depending on the target receptor and cell involved. (A, B) The absence of FcγRIIB cytoplasmic domain reflects the fact that this can take place independent of this domain. C) A mAb-opsonized target cell is shown, interacting with activatory FcγR and inhibitory FcγRIIB co-expressed by an immune cell (e.g., macrophage). Interaction with FcγRIIB may compete with activatory FcγRs for mAb Fc binding, and/or inhibit activatory FcγR downstream signaling.