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The Complex Association of FcγRIIb With Autoimmune Susceptibility

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FcγRIIb is the only inhibitory Fc receptor and controls many aspects of immune and inflammatory responses. The observation 19 years ago that *FcγRIIb*$^{-/-}$ mice generated by gene targeting in 129 derived ES cells developed severe lupus like disease when backcrossed more than 7 generations into C57BL/6 background initiated extensive research on the functional understanding of this strong autoimmune phenotype. The genomic region in the distal part of Chr1 both in human and mice in which the *FcγR* gene cluster is located shows a high level of complexity in relation to the susceptibility to SLE. Specific haplotypes of closely linked genes including the *FcγRIIb* and *Slamf* genes are associated with increased susceptibility to SLE both in mice and human. Using forward and reverse genetic approaches including in human GWAS and in mice congenic strains, KO mice (germline and cell type specific, on different genetic background), knockin mice, overexpressing transgenic mice combined with immunological models such as adoptive transfer of B cells from Ig transgenic mice the involved genes and the causal mutations and their associated functional alterations were analyzed. In this review the results of this 19 years extensive research are discussed with a focus on (genetically modified) mouse models.

Keywords: SLE, systemic lupus erythematosus, autoimmue disease, mouse model, Fcgamma receptor IIB, reverse genetics

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INTRODUCTION

Antibodies (Ab) form immune complexes (IC) with their cognate antigen (Ag). IgG-ICs are potent activators of the immune system via cross-linking of receptors for the Fc part of IgG, FcγR, mainly expressed on the surface of cells of the innate immune system.

FcγRs belong to the Ig supergene family of leukocyte FcR and are transmembrane glycoproteins containing a ligand-binding α subunit with two or three extracellular Ig-like domains, a transmembrane and a cytoplasmic domain. In mice, the high-affinity FcγRI, binding monomeric IgG, and the low-affinity receptors for complexed IgG, FcγRIII, and FcγRIV are activating receptors. The α subunits of the activating receptors form a multi-subunit complex with a dimer of the common γ-chain (FcRγ) (1, 2) with an immunoreceptor tyrosine-based activation motif (ITAM). Cross-linking activating FcRs by IC initiates signal transduction via recruitment and subsequent activation of intracellular tyrosine kinases (3), switching on a large variety of effector mechanisms activating inflammatory cascades.

In humans, there are four activating FCGRs. The high-affinity FCGR1 (CD64) and the low-affinity FCGR3A (CD16A) are associated with the common γ chain whereas the low-affinity FCGR2A (CD32A), containing an ITAM in its cytoplasmic domain, and the low-affinity FCGR3B (CD16B), with a glycosylphosphatidylinositol (GPI) anchor, are single-chain receptors. All human

FCGR genes are clustered at the distal end of Chr1, a region associated with susceptibility to autoimmune diseases such as Systemic Lupus Erythematosus (SLE) (4). In mice the FcγRII, -III, and -IV genes are clustered at the distal end of Chr1, a region orthologous with SLE associated genomic intervals on human Chr1 and associated also with susceptibility to autoimmune disease (Lupus-like disease). *FcγRI* is located on Chr3 due to a translocation during evolution after mouse and human had diverged.

In both humans and mice, the activating FcγRs are counterbalanced by one inhibitory single-chain low-affinity receptor FcγRIIb (FCGR2B or CD32B) with an inhibitory motif named immunoreceptor tyrosine-based inhibition motif (ITIM) within its cytoplasmic domain. In addition, co-engagement of FcγRIIb and the ITAM containing B-cell receptor (BCR) on B cells forms an important negative feedback mechanism to control antibody production. This regulatory mechanism of cellular activation by the ITAM-ITIM motif pair, observed originally with FcγR, has been described for many other receptors in the immune system e.g., T cell receptors and B cell receptors (5, 6). This review focuses on the important but still puzzling immune regulatory role of the inhibitory FcγRIIb and the complex association of its impaired function with autoimmunity as studied extensively in mice.

GENERAL CHARACTERISTICS OF FcγRIIb

Isoforms

In humans and mice, there are two membrane-bound isoforms of FcγRIIb identified: FcγRIIb1 and b2 (7) resulting from alternative splicing. The cytoplasmic domain is encoded by three exons whose 5′ exon encodes a 47 amino acid motif that prevents coated pit localization, which inhibits FcγRIIb mediated endocytosis of soluble immune complexes. This exon is present in the mRNA that encodes the b1 isoform, the only isoform expressed on B cells, but absent in the mRNA that encodes the b2 isoform (8, 9) expressed on most innate immune cells. The ITIM dependent inhibition of cell activation is the same for both isoforms. Therefore, the name FcγRIIb is used in this review without making a distinction between the b1 and the b2 isoform.

Expression

In mice FcγRIIb is expressed on all innate immune cells and is the only FcγR expressed on B cells, including pre-, pro-, and mature B cells, memory B cells, plasma cells (10, 11) and B1 cells (12). Unlike many other B cell surface receptors, expression of FcgRIIb is not downregulated during plasma cell differentiation (10). FcγRIIb expression is modulated on different B cell subsets (11) and increases when the B cells become activated (11, 13). T cells do not intrinsically express FcγRs (14). However, it has been reported that expression of FcγRIIb but not any other FcγR, is upregulated in memory $CD8^+$ T cells after *Listeria monocytogenes* infection and tempers the function of these cells *in vivo* (15). Guilliams et al. showed that according to the microarray expression values extracted from public data sets the mRNA expression of FcγRIIb in mice is from high to low as follows: Inflammatory macrophages (Mφ), $Ly6C^{hi}$ classical monocyte, inflammatory monocyte-derived dendritic cell (moDC), lung $CD11b^+$ conventional or classical DC (cDC), $Ly6C^{lo}$ patrolling monocyte, alveolar Mφ, follicular B cell, GC B cell, skin-draining lymph node $CD11b^+$ cDC, spleen $CD8^+XCR1^+$ cDC, spleen plasmacytoid DC (pDC), spleen $CD11b^+$ cDC, neutrophils, spleen Mφ, and NK cells (16). The overall FcγRIIb expression pattern is similar in mouse and human. In mouse cDCs the relatively low expression of FcγRIIb is higher than that of any activating FcγR.

FcγRIIb expression, relative to that of activating FcγRs, is tightly regulated. In mice, C5a rapidly down-regulates FcγRIIb on alveolar Mφ and upregulates FcγRIII on these cells (17, 18). IL-4 downregulates FcγRIIb expression on mouse activated B cells (13, 19). IFNγ increases FcγRIIb expression on B cells (19) and increases the expression of activating FcγR on myeloid effector cells in mice. In humans the Th2 cytokines IL-4, IL-10, and TGF-β increase FCGR2B expression and decrease activating FCGR expression on myeloid cells (20–22) whereas IFNγ decreases FCGR2B expression on these cells and increases activating FCGR expression (23).

FcγRIIb is also expressed on non-hematopoietic cells. Its expression is induced on FDC upon antigen stimulation (24). It has been calculated that almost 70% of total mouse body FcγRIIb is expressed on liver sinusoidal endothelial cells (LSEC) (25, 26). On mouse glomerular mesangial cells, TNFα/IL-1β upregulates FcγRIIb expression whereas IFNγ downregulates FcγRIIb expression and upregulates the activating FcγR (27).

Cellular Function

Co-aggregation of the inhibiting ITIM containing FcγRIIb with activating ITAM containing FcRs results in the recruitment of the inositol polyphosphate-5-phosphatase SHIP1 that counteracts the signals mediated by activating FcRs (3, 28). Therefore, FcγRIIb has a strong regulatory role in all the processes in which activating FcγR are involved. The ratio between activating and inhibiting signals determines the outcome of the cellular response to IgG-ICs. This ratio depends mainly on the following factors: (a) the relative affinities of the different antibody isotypes involved for the different FcγR, (b) the level of opsonization, and (c) the relative expression level of inhibitory and activating FcγR, which is partially determined by the cytokine milieu. The binding of FcγRIIb for IgG-IC is strongest for IgG1 and weakest for IgG2a. So, FcγRIIb expression has the highest impact on IgG1-IC. In addition, FcγRIIb can inhibit complement-mediated inflammation when co-engaged with Dectin-1 by galactosylated IgG1-ICs (29) indicating that its immune-modulatory function in the efferent response is not restricted to the regulation of activating FcγRs.

In B cells co-crosslinking of the BCR and FcγRIIb results in the inhibition of activation, proliferation, Ag internalization and Ab secretion (30–32). Moreover, *in vitro* studies have shown that FcγRIIb on B cells can induce apoptosis upon clustering (10, 12, 28, 33, 34).

FcγRIIb can also function as an endocytic receptor of small ICs. The endocytic properties of FcγRIIb depend on the presence of a di-leucine motif in the intracellular domain (8) and are independent of the ITIM.

Role in Different Tissues and Cell Types

Myeloid Effector Cells

In the efferent phase, FcγRIIb sets a threshold for the activation by IgG-IC of myeloid effector cells, e.g., monocytes, Mφs, and neutrophils. Crosslinking of activating FcγR by IgG-ICs induces effector mechanisms of these cells e.g., soluble IC clearance, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), the release of inflammatory mediators, degranulation, superoxide production, enhancement of Ag presentation, and cell maturation and proliferation. This includes also the regulation of high-affinity IgE receptor-mediated mast cell activation (35).

Lupus-prone (NZBxNZW)F1 mice deficient for the FcR γ chain, lacking functional activating FcγR, do not develop IC-mediated severe glomerulonephritis (GN), despite high autoantibody titers (36). This suggests that FcγR play a dominant role in the efferent phase of Ab-driven diseases including lupus-like disease and therefore FcγRIIb might have a strong protective role in such a disease. In addition, FcγRIIb might also inhibit an ongoing auto-Ab response by suppressing the activating FcγR dependent, IgG-IC-triggered release of inflammatory mediators and other immune regulatory molecules by myeloid effector cells.

Dendritic Cells

DCs are central regulators of immunity determining whether tolerance is induced, or an effective adaptive immune response is generated, bridging innate and adaptive immunity (37–39). DCs have the unique capacity to take up exogenous Ag via a variety of mechanisms and surface molecules, including FcγR, and subsequently process and present the Ag-derived peptides in their MHC molecules to prime naïve T cells. Three main subsets of DCs can be recognized, cDC, moDC and pDC. Their ontogeny and functions have been reviewed extensively (40, 41).

A series of observations suggest that FcγR on cDCs and moDCs can play a role in priming and regulation of adaptive immunity (16). Ag-specific IgG enhances Ab responses to soluble protein Ag via activating FcγRs, probably by increasing Ag presentation by dendritic cells to Th cells (42). Many laboratories have shown that soluble IgG-ICs strongly enhance cross-presentation by using either *in vitro* assays (43–45), or *in vivo* assays with *in vitro* loaded DCs from WT and FcγR KO mice (46–50). Signaling through the activating FcγRs results in lysosomal targeting of the Ag and importantly activation and maturation of the DCs (44), required for their migration to the lymph node and their presentation of Ag-derived peptides in MHC class I to $CD8^+$ T cells (49, 51). In mouse bone marrow-derived DCs (BMDCs), activating FcγRs modulate the expression of many genes, associated with T cell response induction, upon crosslinking by IgG-ICs. This is strongly regulated by FcγRIIb, setting a threshold for DC activation and maturation (52). *FcγRIIb*$^{-/-}$ mice showed an increased upregulation of costimulatory molecules, resulting in an enhanced capacity to generate antigen-specific T cell responses upon injection of IgG-ICs (52–54). However, *in vivo*, in mice, the role of FcγR in the presentation of soluble IgG-IC derived Ag is redundant (55, 56). In mice, cDCs consist of two main subsets, type 1 cDC or cDC1 and Type 2 cDC or cDC2 (41). *In vivo* IgG-IC improve strongly cross-presentation of the cDC2 but not the cDC1 DCs. Only cDC2 mediated cross-presentation is FcγR dependent (57). Moreover, FcγRs are dispensable for the *in vivo* uptake of IgG-IC by cDC1 and cDC2 (56, 57). The *in vivo* cross-presentation of IgG-IC derived Ag by cDC1 is completely and by cDC2 partially dependent on C1q (56).

Because it has been shown that treatment with FCGR2B blocking antibodies results in spontaneous maturation of human DCs (58) it has been hypothesized that FCGR2B does not only regulate DC activation but also actively prevents unwanted spontaneous DC maturation by small amounts of circulating IC present in serum under non-inflammatory steady-state conditions (2).

IgG-ICs endocytosed by activating FcγR on DCs ends up in a degradative Lamp-1 positive compartment where it is slowly degraded into peptides (59). In contrast, antigen, endocytosed in the periphery via FcγRIIb on DCs, enters preferentially in a non-degradative Lamp-1 negative intracellular vesicular compartment, that recycles to the cell surface to transfer the native antigen via interaction with the BCR to B cells in the lymphoid organs. This indicates that DCs, migrating into extrafollicular areas (60) and the splenic marginal zones (MZ) (61), are not only important for the production of B cell activating components but also for the delivery of Ag to the BCR (62).

The question is whether in an autoimmune disease self-antigen containing IgG-IC can trigger DCs to promote autoreactive immune responses by presenting autoantigens or to release B and T cell activating cytokines and other stimulating factors breaking tolerance and whether FcγRIIb on DCs negatively regulates these processes. That is any way at a stage of the disease that some autoantibodies are already produced.

pDCs produce type I IFN in response to viral nucleic acids sensed through TLR7 and TLR9 (63, 64). Their main function is to control tolerance in the steady state (65, 66). Mouse pDCs express exclusively FcγRIIb (67). Conflicting results have been published regarding FcγRIIb facilitated T cell priming by mouse pDCs (56, 67, 68). *In vitro* uptake of IgG-ICs by mouse pDC is FcγRIIb dependent but does not promote Ag presentation to T cells (67), similarly to what has been shown with FcγRIIb mediated IC uptake in cDCs (62). In contrast, it has been reported that subcutaneous (s.c.) injection of *in vitro* IgG1-IC loaded pDCs induces strong Ag-specific $CD4^+$ and $CD8^+$ T cell responses although with lower efficiency than cDCs. The IgG1-IC-loaded pDC mainly promoted a Th2/tolerogenic environment *in vivo* (68). Human pDCs express besides low levels of FCGR2B, the activating FCGR2A and FCGR3B (16) and show FCGR2A dependent IgG enhanced Ag presentation to T cells (69). SLE patients have circulating ICs, containing small nuclear RNA and anti-small nuclear RNA IgG. pDCs can acquire such IC via FCGR mediated uptake resulting in stimulation of TLR7 and 8 and production of IFNα (70), a cytokine that is believed to play a central role in SLE pathogenesis (71). However, this requires FCGR2A and not FCGR2B (72). Therefore, it is

unlikely that such a pathogenic process plays a role in lupus-like disease in mice.

B Cells and FDC

Primary B cells, developed and selected in the bone marrow, are recruited into GCs within the spleen and lymph nodes to undergo affinity maturation by Somatic Hypermutation (SHM). Three main mechanisms maintain self-tolerance in the primary B cell repertoire: central clonal deletion, receptor editing, clonal anergy induction (73). The first two effectively remove autoreactive B cells from the system. Clonal anergy occurs when self-reactive B cells interact with a self-Ag with relatively low avidity. The result is that BCR signaling is desensitized because of chronic exposure to self-antigens (74, 75) and differentiation into plasma cells is suppressed (76) resulting in the maintenance of anergic B cells with the potential to produce auto-Abs which can be recruited into GC (77). Anergic B cells can get T help if their BCR cross-reacts with foreign Ag but because of impaired BCR signaling FAS-mediated apoptosis is induced. However, extensive cross-linking by a foreign antigen can overcome the attenuated BCR signaling in anergic B cells inhibiting apoptosis (74). Autoreactive primary B cells can escape negative selection because of "clonal ignorance" when self-reactive B cells cannot encounter their self-Ag because it is hidden inside the cell. Development, responsiveness, and lifespan of ignorant cells is normal (76, 78, 79). The lack of T cell help after Ag contact induces apoptosis in ignorant self-reactive B cells in the periphery. However, it is striking that many auto-Abs are directed against intracellular Ags such as DNA. Therefore, it has been suggested that ignorant self-reactive B cells might be important for the development of SLE (77). So, the GC has to deal with three types of potential autoreactive B cells: anergic and ignorant, both recruited, and newly generated by somatic hypermutation in the GC reaction. Several mechanisms are in place in the GC to avoid the development of auto-Ab producing plasma cells. A very high concentration of self-Ag in the GC either overrules the binding of the BCR to foreign Ag presented by the FDC and apoptosis is induced, because of the lack of additional signals provided by the FDC (80), or/and blocks presentation of foreign Ag to follicular helper T cells (T_{FH}), whose survival signals are required. Alternatively, self-reactive B cells can be maintained temporarily until their self-reactivity is abrogated by somatic hypermutation (SHM) (81). Ignorant self-reactive primary B cells, activated by cross-reactive foreign Ag, can enter the GC to get T_{FH} help (82) and subsequently, receptor editing by SHM can destroy self-recognition and improve specificity for foreign antigen. However, this appears not sufficient to prevent that autoreactive B cells escape negative selection in the GC and enter the AFC (antibody-forming cell) pathway. More downstream tolerance checkpoints are required.

In the GC Ag is presented to B cells on the cell surface of FDC, mainly in the form of CR1/2 bound C3d-coated ICs. FcγRIIb is expressed on both the GC B cell and the FDC. Although FcγRIIb is upregulated on FDC in GC compared to non-GC FDC, its expression is relatively low compared to CR2 expression. Therefore, it is unlikely that FcγRIIb plays a role in the capture and presentation of Ag early on in the GC response (83). It is unclear how a GC B cell becomes activated, because binding of its BCR to the Ag, within the FDC bound ICs, will also crosslink FcγRIIb on that B cell. It has been suggested that FcγRIIb expression on FDC competes with FcγRIIb expression on GC B cells by binding most of the Fc domains in the ICs (84). The outcome of co-engagement of BCR and FcγRIIb by ICs bound to FDC in GC might be dependent on the balance between concurrent activating and inhibiting signals, leading to stimulatory, inhibitory, or apoptotic responses (33, 85, 86). FcγRIIb might set a threshold for B-cell activation, that enables the selection of B cells with a BCR with sufficiently high affinity, to become activated. B cells with BCRs that have lost their affinity for the presented Ag during the process of affinity maturation by SHM will get only signals via crosslinking of FcγRIIb, which could result in induction of apoptosis as has been demonstrated *in vitro* (28, 33, 87, 88). In conclusion, the inhibitory FcγRIIb would be an important checkpoint for the deletion of potentially autoreactive B cells in the GC.

An additional apoptosis inducing mechanism in the bone marrow might also contribute to the control of autoreactive B cells (10). Long-lived plasma cells persist in the bone marrow. To provide room to newly generated plasma cells that migrate to the bone marrow after a new infection has occurred, a restricted number of plasma cells in the bone marrow has to be eliminated. Based on observations *in vitro* and *in vivo* in mice it has been hypothesized that plasma cells (which intrinsically lack BCR expression) are killed by apoptosis, induced by cross-linking of FcγRIIb highly expressed on these cells (10).

Non-immune Cells

On LSEC FcγRIIb might function as an endocytic scavenger receptor removing small IgG-IC from circulation to prevent systemic IC triggered inflammation (25). FcγRIIb on renal mesangial cells might protect against IgG-IC induced inflammation in the kidney (89). Both mechanisms might protect against the pathogenesis of IC-driven autoimmune diseases such as glomerulonephritis in SLE in the efferent phase. Because of the lack of an endothelial cell-specific Cre expressing strain that is not transcriptionally active during early hematopoiesis, required to generate endothelium-specific FcγRIIb deficient mice, the specific role of FcγRIIb on LSEC should be studied by applying transplantation of bone marrow from WT mice into lethally irradiated *Fcγ RIIb* KO mice.

FORWARD GENETICS: ASSOCIATION OF AUTOIMMUNITY AND FcγRIIb POLYMORPHISM

In Mice

The association between autoimmunity and *Fcγ RIIb* polymorphism is extensively studied in NZW and NZB inbred stains. NZB mice show limited autoimmunity (90) while NZW mice are not autoimmune although their B cells have intrinsic defects sufficient to break tolerance to nuclear antigens (91, 92). However, the (NZBxNZW)F1 offspring of an accidental cross between NZW and NZB mice (93) showed a severe lupus-like

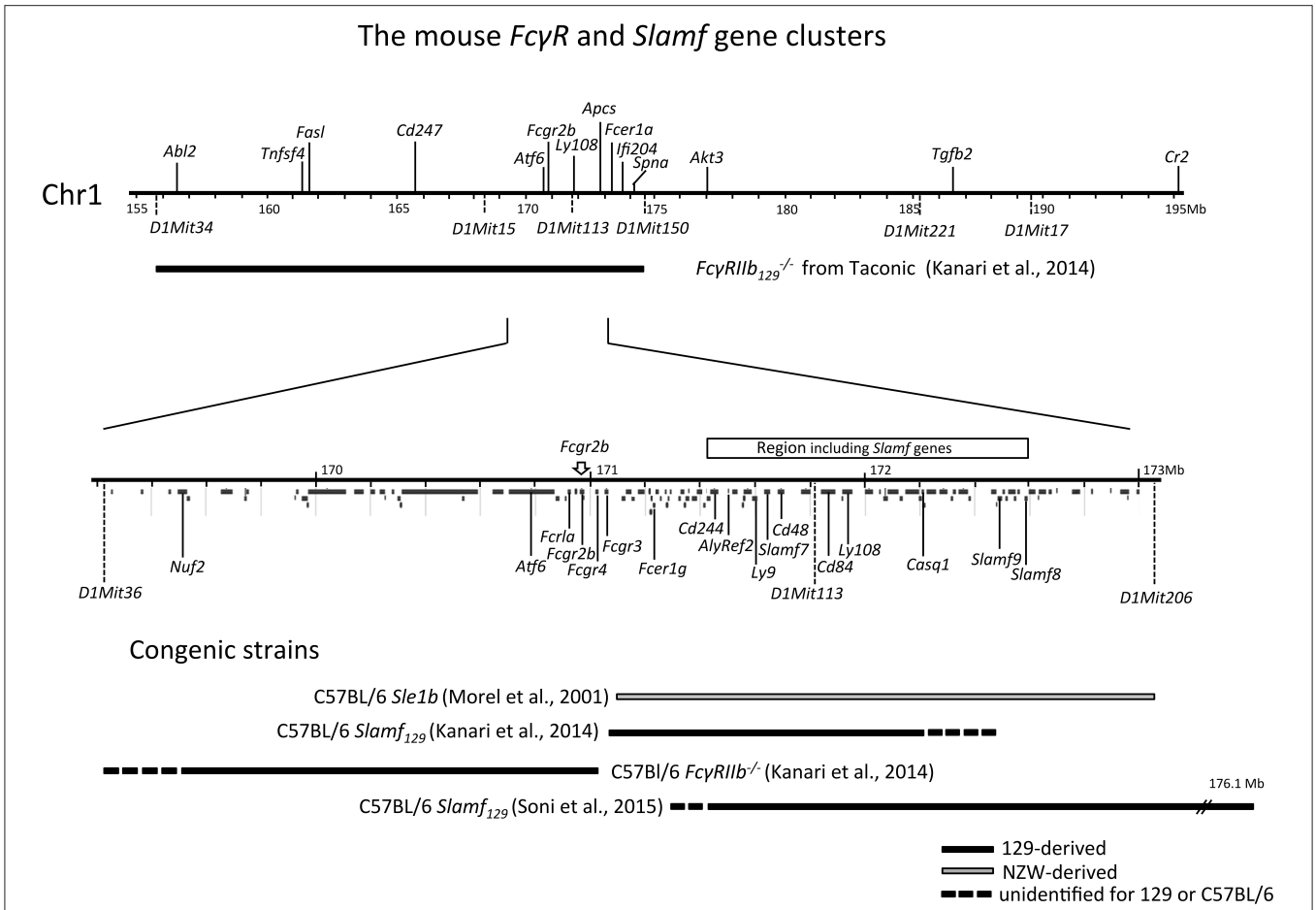

FIGURE 1 | Physical map of the sub-telomeric region of mouse chromosome 1. The upper horizontal line represents a 40 Mb genomic region including the 129 derived *FcγRIIb* flanking region present in the original $FγRIIB^{-/-}_{129}$ mouse, backcrossed more than 7 generations into C57BL/6 background. This *FcγRIIb* flanking region spans at a minimum the distance between the microsatellite markers *D1Mit34* and *D1Mit150* [horizontal black bar; (98)]. The lower horizontal line represents a magnification of the 3.8 Mb subregion located between microsatellite markers *D1Mit36* and *D1Mit206* showing a detailed map of the *FcγR* and *Slam* family gene clusters within this region. In addition, below the line, the location of all other coding genes in this region is shown according to the NCBI database. At the bottom, the congenic fragments present in the different $FcγRIIb^{-/-}$ and $Slamf_{129}$ C57BL/6 congenic strains, described in the text, are depicted as horizontal bars.

phenotype characterized by a gender-bias, expansion of activated B and $CD4^+$ T cells, splenomegaly, elevated serum ANA and IC-mediated GN causing renal failure and premature death at 10–12 months of age (94). By backcrossing (NZBxNZW)F1xNZW followed by brother-sister mating the NZM2410 recombinant inbred strain with a homozygous genome was generated (95–97). In this mouse four SLE susceptibility loci, *Sle1-4*, have been identified on different chromosomes. *Sle1* is located on the telomeric region of Chr1 syntenic to human 1q23 that has shown strong linkage to SLE susceptibility in all human studies. The *FcγR* gene cluster maps in this region (**Figure 1**) and is from NZW origin in NZM2410 mice. From the NZM2410 strain, C57BL/6 strains have been developed congenic for a single SLE susceptibility locus. The presence of *Sle1* appeared to be sufficient to break tolerance in C57BL/6 mice and to drive the production of high titers of anti-chromatin ANAs with a selective Ab reactivity to H2A/H2B/DNA sub-nucleosomes (99, 100). Importantly, this step appears to be necessary for the induction of disease (100) making *Sle1* a key locus in the initiation of SLE. Transplantation of hematopoietic stem cells from C57BL/6 *Sle1* congenic mice into C57BL/6 recipient mice showed that *Sle1* causes independent B and T cell-intrinsic effects on the B cell response (101, 102).

Three *FcγRIIb* haplotypes [numbered I-III according to Jiang et al. (103), **Table 1**] have been recognized in inbred strains of mice and wild mice with variation in the promoter region and intron 3 (**Table 1**). Haplotype I with 2 deletions in the promoter region and one in intron 3 is found in autoimmune-prone strains and most wild mice and is associated with decreased expression of FcγRIIb on Mφ, activated B cells and GC B cells (11, 103–105). By using C7BL/6 congenic strains with the NZW (106) and NZB (107) allelic variants of *FcγRIIb* the effect of the deletions in haplotype I and II on B cell expression was studied. When immunized with KLH, FcγRIIb expression on splenic non-GC B

TABLE 1 | Allelic variants of mouse *FcγRIIb* gene and their association with impaired expression and autoimmune disease susceptibility.

Haplotype	Mouse strain	Genetic variation	Phenotype
I	NZB, BXSB, MRL, NOD, Wild mice 129	13 bp 5′ deletion in promoter 3 bp 3′ deletion in promoter 4 bp 5′ deletion in intron 3	Decreased expression on Mφ and activated and GC B cells. Autoimmune-prone (except 129)
II	NZW, SWR, SJL	4 bp 5′ deletion in intron 3 24 b 3′ deletion in intron 3	Decreased expression on GC B cells. Potential to accelerate autoimmunity
III	C57BL/6, BALB/c, DBA	No deletions	Not autoimmune

cells was high and similar in C7BL/6 and C57BL/6 $Fc\gamma RIIb_{NZB}$ congenic mice. In contrast, the expression on activated GC B cells was markedly down-regulated in C57BL/6 congenic $Fc\gamma RIIb_{NZB}$ mice and up-regulated in control C57BL/6 mice, in comparison with the expression levels on non-GC B cells. The downregulation of FcγRIIb expression on activated GC B cells was associated with an increase of IgG anti-KLH Ab titers. C57BL/6 $FcgRIIb_{NZB}$ congenic mice also showed lower FcγRIIb expression on Mφ compared with WT C57BL/6 mice (107). In a C57BL/6 knockin (KI) mouse model of the 5' region of the haplotype I $Fc\gamma RIIb$ gene ($Fc\gamma RIIb_{NZB}$), FcγRIIb failed to be upregulated on activated and GC B cells resulting in enhanced early GC responses and low auto-Ab production without kidney disease as discussed later in more detail (11).

As mentioned earlier, *in vitro* cross-linking of FcγRIIb on B cells from C57BL/6 mice can induce apoptosis. However, plasma cells from autoimmune-prone NZB or MRL mice could not be killed *in vitro* by FcγRIIb cross-linking because of too little expression of the receptor (10). This might partially explain why these autoimmune-prone mice have larger numbers of plasma cells and might contribute to the autoimmune phenotype of these mice.

Similarly, to the $Fc\gamma RIIb_{NZB}$ allele, the $Fc\gamma RIIb_{NZW}$ allele in the C57BL/6 *Sle1* congenic strain did not upregulate its expression on GC B cells and plasma cells, as did the C57BL/6 allele, when immunized with SRBCs. However, in the absence of its *Sle1* flanking regions, $Fc\gamma RIIb_{NZW}$ did not induce an autoimmune phenotype but was associated with an increased number of class-switched plasma cells (108). This might indicate that the decreased expression of the $Fc\gamma RIIb_{NZW}$ allele is not sufficient for the development of autoreactive B cells but can result in the increase of the number of autoreactive B cells, induced by other lupus-susceptibility loci, by enhancing the production of class-switched plasma cells. This suggests that the $Fc\gamma RIIb_{NZB}$ (haplotype I) allele has a stronger impact on susceptibility to autoimmunity than the $Fc\gamma RIIb_{NZW}$ (haplotype II) allele (**Figure 2**). However, in one study comparing the phenotypes of C57BL/6 strains congenic for different intervals of the *Nba2* locus, a region on Chr1 of NZB mice corresponding to the $Sle1_{NZW}$ locus, $Fc\gamma RIIb_{NZB}$ was identified as an autoimmune susceptibility gene (114), in another it was not (115). *Sle1* can be divided in four non-overlapping sub-loci: *Sle1a, -b, -c*, and *-d. Sle1b* is far the most potent autoimmune susceptibility locus causing almost the same phenotype as the whole *Sle1* locus: gender-biased spontaneous loss of immune tolerance to chromatin, the production of high titers of IgG auto-Abs with a penetrance of 90% at 9 months of age and increase of total IgM and B7-2 expression on B cells (116). This suggests that *Sle1b* mainly affects B cells. The genomic location of *Sle1b* was determined by phenotypic analysis (e.g., ANA production) of a series of C57BL/6 congenic strains carrying truncated *Sle1* intervals. C57BL/6 congenic mice with an NZW derived genomic fragment, containing the $Fc\gamma R$ cluster, did not develop ANA whereas C57BL/6 mice, containing an adjacent 900 kb congenic NZW fragment expressing 24 genes including seven members of the highly polymorphic signaling lymphocytic activation molecules (*Slam*) cluster, did. This positions the $Fc\gamma R$ cluster just outside the *Sle1b* locus (117) and confirms previous observations that $Fc\gamma RIIb$ is located in between the *Sle1a* and *Sle1b* loci (113) (**Figure 1**). Together these data suggest that in C57BL/6 *Sle1* congenic mice the $Fc\gamma RIIb_{NZW}$ allele is not required for the development of an autoimmune phenotype, whereas the adjacent *Slam* cluster is. Because of these puzzling results, the questions remain why FcγRIIb is upregulated on GC B cells in non-autoimmune inbred strains such as C57BL/6 and BALB/c and why this is impaired in autoimmune-prone mouse strains and how does that contribute to the autoimmune phenotype of these mice.

Slam family (*Slamf*) member genes encode cell surface glycoproteins with extracellular binding domains that mediate stimulatory and/or inhibitory signaling via associations with members of the Slam-associated protein (SAP) family of signaling adaptors during cell-cell interactions between many hematopoietic cell types (118–120). They are the only genes within the *Sle1b* interval with obvious immunological functions (117). Most Slamf members act as self-ligand and are expressed on many lymphoid and myeloid cell subsets, platelets, and hematopoietic stem and progenitor cells. Slamf plays a role in the interaction of $CD4^+$ T cells with cognate B cells, recruitment and retention of T cells within the emerging GCs (121–123), long-lasting T cell:B-cell contact, optimal T_{FH} function, T cell activation (124, 125), stabilization of B–T cell conjugates and sustaining effective delivery of T cell help required for GC formation (126, 127).

The *Slamf* genes show extensive polymorphisms (117) but only two haplotypes of the *Slamf* locus have been identified in laboratory mouse strains. Haplotype 1 is represented by C57BL/6 and related strains and haplotype 2 by all autoimmune-prone mouse strains, as well as many non-autoimmune mouse strains including BALB/c and 129. The polymorphism in *Slamf* member *Ly108* affects the expression of two alternatively spliced isoforms, Ly108-1 and, Ly108-2, which differ exclusively in their cytoplasmic region (117). Ly108-1 is dominantly expressed in T and B lymphocytes of mice with haplotype 2, whereas Ly108-2 is dominantly expressed in T and B cells of mice with haplotype 1. Modulation of the BCR signaling by Ly108-1 results in the impaired negative selection of B cells (128). Overexpression of

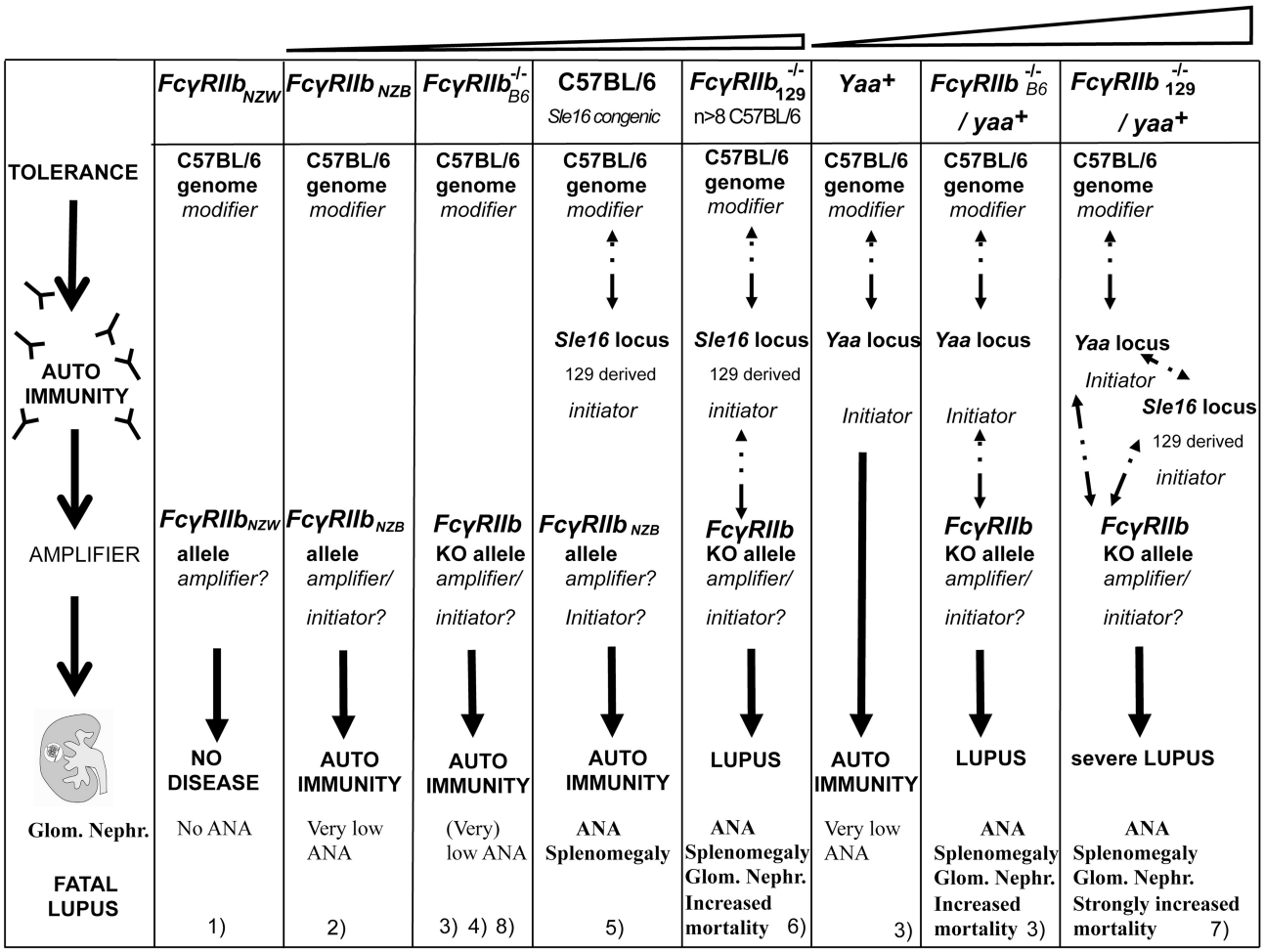

FIGURE 2 | Epistasis between the *FcγRIIb* KO alleles and the *Sle16 (Slam*$_{129}$*)* and *Yaa* autoimmune susceptibility loci resulting in lupus-like disease in C57BL/6 mice. Epistatic interactions are indicated as dotted arrows. The FcγRIIb flanking *Sle16* genomic region contains the autoimmunity associated *Slamf*$_{129}$ haplotype 2 gene cluster (see **Figure 1**). (1) Rahman et al. (108); (2) Espéli et al. (11); (3) Boross et al. (109); (4) Li et al. (110); (5) Bygrave et al. (111); (6) Bolland and Ravetch (112); (7) Bolland et al. (113); (8) Kanari et al. (98). The increasing severity of autoimmune disease in the different mouse models is depicted on top.

both C57BL/6 derived non-autoimmune Ly108 and CD84 Slamf members was required to restore tolerance in autoimmune-prone C57BL/6 *Sle1* congenic mice (129), indicating that polymorphism in both *Slamf* genes contributes to the autoimmune phenotype of C57BL/6 *Sle1* congenic mice.

In the NZM2410 model four NZW-derived SLE suppressor loci have been identified (130). The presence of such suppressor loci might explain why NZW and also129 and BALB/c mice do not develop autoimmune disease, although they carry the type 2 *Slamf* haplotype.

In Humans

The reported copy number variation (CNV) in human *FCGR* genes does not involve *FCGR2B* (131–134). A series of single nucleotide polymorphisms (SNPs) have been reported to be located both in the promoter and the encoding region of the human *FCGR2B* gene (135). Two SNPs are located in the promoter region at nucleotide positions−386 and −120 (−386G>C; *rs3219018* and −120A>T; *rs34701572*) (136) resulting in four haplotypes:−386G−120T (named *FCGR2B.1*), −386C−120T (*FCGR2B.2*),−386G−120A (*FCGR2B.3*), and −386C−120A (*FCGR2B.4*). The rare *FCGR2B.4* haplotype increased the transcription of *FCGR2B in vitro* and resulted in increased FCGR2B expression on EBV transformed B cells and primary B cells (137) and myeloid cells (138), compared to the more frequent *FCGR2B.1* haplotype. However, independently, others have shown that homozygosity of the −386C genotype decreases the transcription and surface expression of FCGR2B in peripheral B cells compared to the −386G homozygote genotype (139). Up till now, there is no explanation for these contradictory results.

In the transmembrane encoding fifth exon a non-synonymous C to T transition was identified, *rs1050501*, resulting in the substitution of isoleucine with threonine at position 232 (140), excluding the receptor from lipid rafts. This prevents interaction of FCGR2B with ITAM containing receptors such as the activating FCGR and the BCR (141, 142). Mφs from individuals homozygous for $FCGR2B^{T232}$ showed a stronger phagocytic capacity of IgG-IC while the B cells of these individuals showed reduced FCGR2B-mediated inhibition of BCR-triggered proliferation (142).

GWAS analyses have shown an association between *rs1050501* and SLE (140, 143–147). Three meta-analyses confirmed these associations (147–149). The $FCGR2B^{T232}$ homozygosity is associated with an odds ratio of 1.73, one of the strongest associations in SLE (147). Association of *rs1050501* with Rheumatoid Arthritis (RA) has been reported for a Taiwanese cohort (150).

The frequency of homozygosity of the $FCGR2B^{T232}$ allele is only 1% in Caucasians and in contrast 5–11% in African and South-East Asian populations (151). This might be one of the explanations for the ethnic differences in SLE susceptibility. Malaria is endemic in Africa and South-East Asia. An association was found between decreased susceptibility for severe malaria and homozygosity for the $FCGR2B^{T232}$ allele (135). So, increased protection against malaria by down-regulation of FCGR2B expression goes along with increased risk to develop SLE.

A significant but weak association has been observed between *SLAMF* and susceptibility to SLE. The weakness of the association might be explained by the limited size of the cohorts studied (152). An association study of UK and Canadian families with SLE has revealed multiple polymorphisms in several *SLAMF* genes (153). However, the strongest association with a non-synonymous SNP could not be replicated in independent Japanese and European cohorts of SLE patients (154, 155). Instead, another SNP was significantly associated with the susceptibility to SLE in another Japanese cohort (156). One large-scale case-control association study showed an association of two SNPs with increased susceptibility to RA, in two independent Japanese cohorts (155). In conclusion, these observations indicate that also in human's polymorphisms of *SLAMF* contribute to the susceptibility to autoimmune disease.

Overall, a model emerges from both studies with C57BL/6 *Sle* congenic mouse strains and human SLE (157), in which disease susceptibility arises through the co-expression of multiple genetic variants that have weak individual effects (152, 158). According to the "threshold liability" model, the severity of the autoimmune phenotype increases with the increasing number of autoimmunity associated allelic variants of autoimmune susceptibility genes in the genome. However, epistatic interactions might result in a more complex non-additive inheritance of the autoimmune phenotype (**Figure 2**). According to this "multiplicative model" the interactions of all susceptibility and suppressor alleles in the genome determine the susceptibility for autoimmune diseases of an individual (159). Importantly this means that the contribution of an individual gene to the autoimmune phenotype can vary depending on the presence of other susceptibility and suppressor genes in the genome (the genomic context). This might explain the puzzling and contradictory results with the $Fc\gamma RIIb_{NZW}$ and $Fc\gamma RIIB_{NZB}$ haplotypes. To uncover the polygenic effects associated with a complex disease such as SLE not a single gene association approach but gene set analysis (GSA) is required (160). However, a reverse genetic approach might offer the opportunity to reconstruct an autoimmune phenotype by modifying a combination of a limited number of candidate genes in a well-defined genetic background.

REVERSE GENETICS

So far three *FcγRIIb* KO mouse models have been published. The first published KO was generated by gene targeting in 129 derived ES cells (161) and subsequently backcrossed into the C57BL/6 background, here called $Fc\gamma RIIb^{-/-}_{129}$ mouse. This mouse on a not well-defined mixed genetic background was during 15 years (between 1996 and 2011) the only *FcγRIIb* KO model available and has been extensively used resulting in an overwhelming amount of literature concerning the role of FcγRIIb in immune tolerance. Subsequently, independently, in two different laboratories *FcγRIIb* KO mice were generated by gene targeting in C57BL/6 derived ES cells, here called $Fc\gamma RIIb^{-/-}_{B6}$ mice (109, 110). The published data regarding ANA titers of one of these mouse strains are inconsistent (162, 163) as are the autoimmune phenotypes of both C57BL/6 strains (109, 110). Moreover, it is still under debate to what extent the autoimmune phenotypes of the $Fc\gamma RIIb^{-/-}_{B6}$ mice differ from the autoimmune phenotype of the $Fc\gamma RIIb^{-/-}_{129}$ mice. Therefore, we discuss in chronological order these different models.

The *FcγRIIb* KO Mouse on Mixed 129/C57BL/6 Background

The $Fc\gamma RIIb^{-/-}_{129}$ mouse develops elevated immunoglobulin levels in response to both T cell-dependent and T cell-independent Ags (161), have more plasma cells (10), and show an enhanced passive cutaneous anaphylaxis compared to WT controls (161). They develop arthritis (164) and Good pasture's syndrome-like disease (165) upon immunization with bovine collagen type II and type IV, respectively when backcrossed into the non-permissive ($H\text{-}2^b$ haplotype) C57BL/6 background. When backcrossed more than 7 generations into C57BL/6, but not BALB/c background, the $Fc\gamma RIIb^{-/-}_{129}$ mice started to develop spontaneously with high penetrance lupus-like disease. This autoimmune disease is characterized by gender bias, splenomegaly, increase of the proportion of different subsets of activated lymphocytes with age, high titers of ANA, IC-mediated GN and vasculitis in different organs resulting in proteinuria and premature death (112) very similar to the phenotype of the NZM2410 mouse we discussed earlier. This is surprising because, as we have seen, genetic studies revealed that lupus susceptibility is a multigenic phenotype. Monogenic autoimmune diseases are rare (158). However, the strong autoimmune phenotype of the $Fc\gamma RIIb^{-/-}_{129}$ mouse cannot be attributed exclusively to the deletion of the *FcgRIIb* alleles. This mouse has been generated by gene targeting in 129 derived ES cells and subsequently

backcrossed into C57BL/6 background. Such a mouse is, even after 10–12 generations, not fully C57BL/6 but congenic for the 129 derived flanking regions of the targeted allele, containing still hundreds of genes of 129 origin (**Figure 1**). The 129 genome contains more than 1,000 non-synonymous mutations compared to the C57BL/6 genome (166). This is only one part of the problem. Epistasis between 129 derived loci and the C57BL/6 genome also occurs. It has been shown that mice without targeted alleles but congenic for the 129 derived distal-region of Chr1 (*Sle16*), a lupus-associated region including the autoimmune-prone haplotype 2 of the *Slamf* genes and the haplotype I of the *FcγRIIb* gene, develop a similar autoimmune phenotype as C57BL/6 *Sle1* congenic mice (111). That might explain why several mouse strains generated by targeting genes in the proximity of the *Slamf* locus in 129 derived ES cells, and backcrossed into C57BL/6 background, develop autoimmunity.

Strikingly, the $FcγRIIb_{129}^{-/-}$ mouse backcrossed more than seven generations into C57BL/6 background develops ANA with similar selective reactivity to H2A/H2B/DNA sub-nucleosomes as C57BL/6 *Sle1* congenic mice, however, with earlier onset, stronger penetrance, and higher titers. Irradiated $Rag^{-/-}$ C57BL/6 or $IgH^{-/-}$ C57BL/6 mice adoptively transferred with bone marrow from $FcγRIIb_{129}^{-/-}$ mice backcrossed more than seven generations into C57BL/6 background developed anti-chromatin antibodies and proteinuria, indicating that the disease is fully transferable, dependent on B cells. Myeloid $FcγRIIb^{-/-}$ cells are not required (112). This is in keeping with experiments, mentioned earlier, that show that the autoimmune phenotype of C57BL/6 *Sle1* congenic mice is completely reconstituted in C57BL/6 irradiated mice that received bone marrow from C57BL/6 *Sle1* congenic mice but not by the reciprocal reconstitution. This demonstrates that *Sle1* is functionally expressed in B cells (101) although impaired FcγRIIb expression seems to play a minor role in that model (113, 117). Taken together these data all point in the same direction: the strong lupus-like phenotype of the $FcγRIIb_{129}^{-/-}$ mice backcrossed more than seven generations into C57BL/6 background is caused by epistatic interaction between the $Slamf_{129}$ locus, the C57BL/6 genome, and $FcγRIIb^{-/-}$ (**Figure 2**), similar to the epistatic interactions between $FcγRIIb_{NZB}$ (haplotype I), $Slamf_{NZB}$ (haplotype 2) and the C57BL/6 genome in C57BL/6 *Nba2* congenic mice (114). As a consequence, the $FcγRIIb_{129}^{-/-}$ mouse suffers from the confounding effect that the $FcγRIIb_{129}$KO alleles are closely linked to the $Slamf_{129}$ locus associated with autoimmunity. This means that in most experimental conditions, no distinction can be made between $FcγRIIb^{-/-}$ and $Slamf_{129}$ mediated effects in these mice.

Ig gene analysis of ANA suggests that ANA develop in GCs (167–172). Therefore, analysis of the loss of tolerance in $FcγRIIb_{129}^{-/-}$ mice focused on GC (173). The role of FcγRIIb as an immune tolerance checkpoint has been studied in a transgenic mouse model in which the variable heavy chain (V_H) *3H9H-56R*, derived from a dsDNA specific hybridoma, or its variant $56RV_H$, with higher affinity binding to dsDNA, were inserted in the *Igh* locus (IgM^a allele) (174). Receptor editing, based on the use of specific light chains that abrogates the dsDNA binding, is the main mechanism to maintain tolerance in these mice (175–177). The Ab selection process was compared between WT C57BL/6 and $FcγRIIb_{129}^{-/-}$ mice carrying the V_H transgenes (178). C57BL/6 mice expressing the high-affinity *56R* allele (B6.*56R*) developed low but significant anti-DNA titers, indicating that tolerance was broken, whereas C57BL/6 mice with the low-affinity *3H9* allele (B6.*3H9*) did not. Tolerance was also maintained in $FcγRIIb_{129}^{-/-}$ mice carrying the low-affinity *3H9* allele ($FcγRIIb_{129}^{-/-}$.*3H9*). The development of IgM-positive autoreactive B cells was similar in $FcγRIIb_{129}^{-/-}$ mice carrying the high-affinity *56R* allele ($FcγRIIb_{129}^{-/-}$.*56R*) and B6.*56R* mice. Moreover, $FcγRIIb_{129}^{-/-}$.*3H9* mice and $FcγRIIb_{129}^{-/-}$.*56R* mice did not show differences in the populations of activated and GC B cells or T cells compared to B6.*3H9* and B6.*56R* control mice. However, $FcγRIIb_{129}^{-/-}$.*56R* mice developed higher IgG anti-DNA titers compared to B6.*56R* mice. Taking together these observations suggest that the function of FcγRIIb in B6.*56R* mice is limiting the production of serum IgG anti-dsDNA. Analysis of hybridomas derived from these different mouse strains showed that a much higher percentage of hybridomas from $FcγRIIb_{129}^{-/-}$.*56R* mice secreted IgG antibodies compared to the hybridomas from B6.*56R* mice. Moreover, $FcγRIIb_{129}^{-/-}$.*56R* mice had a higher percentage of splenocytes with a plasma cell phenotype compared to B6.*56R* mice. The cross of the $FcγRIIb_{129}^{-/-}$ mice with autoimmune B cell receptor transgenic mice most likely bypasses the involvement of $Slamf_{129}$ (which is mainly responsible for the spontaneous development of autoreactive B cells in a C57BL/6 $Slamf_{129}$ congenic strain, as we will see later). So, in this case, the phenotype of the $FcγRIIb_{129}^{-/-}$.*56R* mouse can be completely attributed to the absence of FcγRIIb. From these results, it was concluded that the main function of FcγRIIb in the GC reaction is to control, as one of the latest checkpoints, the development of autoreactive IgG-secreting plasma cells and that most likely FcγRIIb deficiency modifies autoimmunity rather than initiates loss of tolerance (178). This was confirmed independently, in an experimental model with two V_H chain knockin strains, HKI65 and HKIR, with specificity for the hapten arsonate and a weak and strong specificity for DNA respectively (179). No indications for a role of FcγRIIb in primary or GC tolerance checkpoints were found. Only an increased number of plasma cells was detected in mice that received C57BL/6 *HKIR*/$FcγRIIb_{129}^{-/-}$ B cells. FcγRIIb seems to prevent autoimmunity by suppressing the production of autoreactive IgG from B cells that escaped negative selection in GC and enter the AFC pathway (179). This is also in agreement with observations in C57BL/6 $FcγRIIB_{NZW}$ congenic mice mentioned earlier (108). However, more recently it has been shown that the number of spontaneous (Spt) GC B cells is increased in 6–7 months old $FcγRIIb^{-/-}$ mice on a pure C57BL/6 background, suggesting that FcγRIIb deficiency dysregulates the Spt-GC B cell response [(163); **Table 3**] as will be discussed later.

The view that FcγRIIb acts as a suppressor of autoimmunity caused by other loci is supported by the observed synergism between $FcγRIIb^{-/-}$ and several autoimmune susceptibility loci. Just like the *Sle1* locus (100), $FcγRIIb^{-/-}$ interacts synergistically

with the autoimmune susceptibility *Yaa* locus from BXSB autoimmune-prone mice, containing the *Tlr7* gene translocated from the X chromosome to the Y chromosome, resulting in strong acceleration of lupus-like disease in $Yaa^{+}Fc\gamma RIIb^{-/-}_{129}$ male mice (113) (**Figure 2**). MRL/$Fas^{lpr/lpr}$ mice develop lupus-like disease whereas C57BL/6 $Fas^{lpr/lpr}$ mice do not, likely due to suppressor activity of the C57BL/6 genome. However, C57BL/6 $Fas^{lpr/lpr}Fc\gamma RIIb^{-/-}_{129}$ mice develop systemic autoimmune disease (180). This is consistent with the presence of the haplotype I allelic variant of *FcγRIIb* in MRL mice with an impaired expression on B cell subsets. Mice deficient for both, deoxyribonuclease 1 like 3 (DNASE1L3) and FcγRIIb exhibit at the age of 10 weeks an IgG anti-dsDNA production higher than in 9 months old (NZBxNZW)F1 mice (181). The presence of either the *Yaa* locus or homozygosity for the Fas^{lpr} or *Dnase1l3* KO alleles is most likely sufficient to break tolerance. However, FcγRIIb prevents strong autoimmunity by suppressing the production of autoreactive IgG from B cells that have escaped negative selection and enter the AFC pathway. Because $Fc\gamma RIIb^{-/-}_{129}$ mice were used in the crosses mentioned a role for $Slamf_{129}$ cannot be excluded in these models as indicated by the much milder phenotype of the Yaa^{+} $Fc\gamma RIIb^{-/-}_{B6}$ mouse on pure C57BL/6 background discussed later (109) compared to the severe lupus phenotype of the $Yaa^{+}Fc\gamma RIIb^{-/-}_{129}$ mouse (**Figure 2**). Nevertheless, these observations underscore the crucial role of FcγRIIb in the protection against the development of spontaneous autoimmunity determined by other autoimmune susceptibility loci.

Because of allelic exclusion, Ig transgenic mice do not have a normal B cell repertoire. Therefore, the development of self-reactive GC B cells and plasma cells was studied in $Fc\gamma RIIb^{-/-}_{129}$ mice by large scale Ig cloning from single isolated B cells to determine how loss of FcγRIIb influences the frequency at which autoreactive ANA-expressing B cells participate in GC reactions and develop in plasma cells under physiological conditions (173). In comparison with WT controls the following was observed in $Fc\gamma RIIb^{-/-}_{129}$ mice: (a) No skewing of *Ig* gene repertoire but enrichment for IgGs with highly positively charged IgH CDR3s which is associated with antibody autoreactivity; (b) lower numbers of somatic mutation; (c) increased numbers of polyreactive IgG^{+} GC B cells and bone marrow plasma cells and (d) enrichment of nucleosome-reactive GC B cells and plasma cells. The overall frequency of ANAs was high in GC B cells but not in plasma cells. These results demonstrate that in $Fc\gamma RIIb^{-/-}_{129}$ mice IgG autoantibodies including ANAs are expressed by GC B cells and that somatic mutations contribute to the generation of high-affinity IgG antibodies suggesting that the $Fc\gamma RIIb^{-/-}$/$Slamf_{129}$ combination plays an important role in the regulation of autoreactive IgG^{+} B cells which develop from non-self-reactive or low-self-reactive precursors by affinity maturation (173). It would be of great interest to repeat this analysis in $Fc\gamma RIIb^{-/-}_{B6}$ mice on pure C57BL6 background and C57BL/6 $Slamf_{129}$ congenic mice to define the individual contribution of the $Slamf_{129}$ locus and the *FcγRIIb* KO alleles in the loss of immune tolerance in the C57BL/6 background. Interestingly the frequency of high-affinity autoreactive IgG^{+} plasma cells was relatively low, given the high frequency of autoreactive IgG^{+} GC B cells. This can be explained by the existence of a tolerance checkpoint before GC B cells differentiate into spleen or bone marrow plasma cells, downstream of FcγRIIb and Slamf (173).

Complementation of the mutant phenotype of an organism by expression of a transduced WT gene is considered as the ultimate proof that the mutated gene is the cause of the phenotype. Irradiated autoimmune-prone BXSB, NZM2410, and $Fc\gamma RIIb^{-/-}_{129}$ mice transplanted with autologous bone marrow transduced with a viral vector expressing FcγRIIb showed reduced autoantibody levels and as a consequence much milder disease symptoms compared to mice that received autologous bone marrow transduced with an empty vector (182). These results were confirmed by using a transgenic mouse with a stable 2-fold B cell-specific overexpression of FcγRIIb (183). These mice hardly developed a lupus-like disease when backcrossed into autoimmune-prone MRL/$Fas^{lpr/lpr}$ background. The underlying mechanism of these strong effects of overexpression of FcγRIIb is not known. These experiments mainly demonstrate that overexpression of FcγRIIb on B cells inactivates these cells resulting in a strong decrease in autoantibody production. Although they confirm a role of FcγRIIb in autoimmune disease they don't answer the intriguing question whether FcγRIIb deficiency is a modifier of autoimmunity rather than a primary initiator of the loss of tolerance.

FcγRIIb KO on a Pure C57BL/6 Background

To avoid the confounding effect of 129 derived flanking sequences (*Sle16*), independently, in two different laboratories $Fc\gamma RIIb^{-/-}$ mice were generated by gene targeting in C57BL/6 ES cells. To distinguish between these two models, one is called here $^{Le}Fc\gamma RIIb^{-/-}_{B6}$ (109) and the other $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ (110). $^{Le}Fc\gamma RIIb^{-/-}_{B6}$ mice exhibit a hyperactive phenotype in the effector phase, although somewhat milder than $Fc\gamma RIIb^{-/-}_{129}$ mice, suggesting a contribution of *Sle16* to the phenotype of the $Fc\gamma RIIb^{-/-}_{129}$ mouse in the effector phase (109). Both KO mice develop very mild lupus-like disease (**Table 2**). Total IgG ANA was not significantly increased in 10 months old female $^{Le}Fc\gamma RIIb^{-/-}_{B6}$ mice compared to C57BL/6 mice although serum of 5% of these mice showed some total IgG anti-dsDNA and anti-ssDNA antibody titers just above (C57BL/6) baseline. In contrast, in 40% of 10 months old $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ mice total IgG anti-nuclear Abs was significantly increased compared to C57BL/6 mice (110). But only five percent of $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ mice showed premature death whereas mortality was not increased in $^{Le}Fc\gamma RIIb^{-/-}_{B6}$ mice although proteinuria and kidney pathology were significantly higher in these mice compared to C57BL/6 mice. The kidney phenotype in the absence of detectable ANA in $^{Le}Fc\gamma RIIb^{-/-}_{B6}$ mice points to a protective role of FcγRIIb in the kidney, in the efferent phase, as has also been shown in a model

TABLE 2 | Disease phenotypes of $Fc\gamma RIIb^{-/-}_{B6}$, C57BL/6 $Fc\gamma RIIB^{-/-}_{129}$ $Slamf_{B6}$ congenic, C57BL/6 $Slamf_{129}$ congenic and the original $Fc\gamma RIIb^{-/-}_{129}$ mice compared to WT C57BL/6 control mice at the age of 6–8 months.

Phenotype \ Mouse	$^{Le}Fc\gamma RIIb^{-/-}_{B6}$ [c]	C57BL6 $Fc\gamma RIIb^{-/-}_{129}$ $Slamf_{B6}$ Congenic[a]	$^{NY}Fc\gamma RIIb^{-/-}_{B6}$ [b,d]	C57BL/6 $Slamf_{129}$ Congenic[a,b]	$Fc\gamma RIIb^{-/-}_{129}$ [a,b,c,d]
Increased IgM	n.d.	–[a]	n.d.	–[a]	+[a]
Increased IgG[a]	n.d.	+ (♀)[a]	n.d.	–[a]	+ (♀ ♂)[a]
α-DNA	+ (♀) Total IgG Incidence 5%[c]	+ (♀) IgG2c[a]	++ (♀) IgG2c[b] Total IgG[d]	+++ IgG2c/2b[b] (♀) IgG2c[a]	+++++++ (♀) IgG2c[a,b] IgG2b[b] Total IgG[a,d]
α-histone	– (♀) Total IgG[c]	n.d.	++ (♀) IgG2c[b]	+++ IgG2c/2b[b]	+++++++ IgG2c/2b[b] Total IgG[c]
α-nuclear	+ (♀) Total IgG[c]	+ (♀) IgG2c[a]	++ (♀) IgG2c[b] Total IgG Incidence 40%[d]	+++ IgG2c[a,b] IgG2b[b]	+++++++ (♀) IgG2c[a,b] IgG2b[b] Total IgG[a,d]
Kidney pathology	+ (♀)[c]	+ (♀)[a]	++[b]	–[a,b]	+++++[a,b,c]
IgG-IC deposition in glomeruli	+ (♀)[c]	+ (♀)[a]	++ (♀)[b]	+ (♀)[b] – (♀)[a]	++++ (♀ ♂)[a,b,c]
C3 deposition	+[c]	n.d.	–[b]	+[b]	+++++[b,c]
Spleen	Slightly enlarged (♀)[c]	Slightly enlarged (♀)[a]	n.d.	Slightly enlarged (♀)[a]	Splenomegaly[a,b,c]
Spt-GC formation	n.d.	Normal (♀ ♂)[a]	Augmented + (♀)[b]	Augmented ++ (♀)[a,b]	Augmented +++ (♀)[a,b]
% GC B cells of $CD19^+$ splenic B cells	n.d.	No increase (♀)[a]	Increase +[b]	Increase ++ (♀)[a]	Increase +++ (♀)[a]
Absolute numbers of splenic GC B cells	n.d.	No increase (♀)[a]	n.d.	Increase + (♀)[a]	Increase ++ (♀)[a]
Increased Mortality	–[c]	–[a]	+ 5%[d]	–[a,b]	Varies from 0%[a] (and 22%[c]) to 60%[d]

[a] *Kanari et al. (98).*
[b] *Soni et al. (163).*
[c] *Boross et al. (109).*
[d] *Li et al. (110).*
n.d., not determined.

of antibody-induced nephrotoxic nephritis (NTN) that will be discussed later (89).

The production of autoantibodies by C57BL/6 mice in the absence of FcγRIIb suggests that FcγRIIb deficiency, besides modifying autoimmunity caused by other autoimmune susceptibility loci (e.g., $Slamf_{129}$, *Yaa*), as discussed earlier, can result in loss of tolerance in the GC. However, it is tempting to speculate that the low titers of autoantibodies, that develop with low penetrance in *FcγRIIb* KO mice on a pure C57BL/6 background, reflect the natural occurring autoreactive B cells in the GC of a WT C57BL/6 mouse, as described earlier, that are prevented to enter the AFC pathway in the presence of FcγRIIb (178). There are indications that C57BL/6 mice are more autoimmune prone than BALB/c mice. For example, B cell receptor editing as a mechanism to maintain B cell tolerance is less effective in these mice compared to BALB/c mice (178).

The $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ mouse seems to exhibit a stronger disease phenotype than the $^{Le}Fc\gamma RIIb^{-/-}_{B6}$ mouse (**Table 2**). There are several explanations for this discrepancy:

a. The strains are generated with different ES cell lines. There might be relevant genomic differences between the C57BL/6 derived ES cell lines used. This question can be answered by sequencing the *FcγRIIb* flanking genomic regions in both mouse strains.
b. The mice have been backcrossed several generations into different C57BL/6 mouse strains. There are substantial genetic variations between the different C57BL/6 strains used in different laboratories (184).
c. Environmental factors (immune status, microbiome) play a role. The incidence of lethal disease in $Fc\gamma RIIb^{-/-}_{129}$ mice varies between different laboratories from 0% to more than 60% (98, 109, 112, 173).
d. Differences in the methods used to measure ANA. In the $^{Le}Fc\gamma RIIb^{-/-}_{B6}$ mouse ANA have been measured only by ELISA of total IgG (109), whereas in the $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ mouse IgG2a and IgG2b have been measured combined with Hep-2 cell staining (163). However, a significant increase in total IgG anti-nuclear Abs compared to C57BL/6 has also been reported with the $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ mouse (110).

TABLE 3 | Characteristics of GC B and T cells in $^{NY}Fc\gamma RIIb^{-/-}_{B6}$, C57BL/6 $Slamf_{129}$ congenic, and the original $Fc\gamma RIIb^{-/-}_{129}$ mice compared with WT C57BL/6 control mice.

Phenotype \ Mouse strain	$Fc\gamma RIIb^{-/-}_{129}$	C57BL/6 $Slamf_{129}$ congenic	$^{NY}Fc\gamma RIIb^{-/-}_{B6}$
Increase in frequency of $B220^{+}PNA^{hi}$ $CD95^{hi}$ Spt-GC B cells	++++	++	+
Increase in Splenic GC size	+++	++	+
Increase in frequency of $CD4^{+}CXCR5^{hi}PD\text{-}1^{hi}$ GC T_{FH} cells	+++	+	–
Increase in frequency of $CD4^{+}CXCR5^{int}PD\text{-}1^{int}$ T_{FH} cells	+++	+	–
Increase in $CD4^{+}GL7^{+}$ GC T_{FH} cells	++	+	–
IL-21 expression in GC T_{FH} cells	++++	++	–
PD-1 expression in GC T_{FH} cells	++++	++	++
ICOS expression in GC T_{FH} cells	++	–	–
Increase in frequency of GC B cells upon antigenic stimulation	n.d.	+	–
Increase in frequency of GC T_{FH} cells upon antigenic stimulation	n.d.	+	–
MHC class II upregulation on GC B cells upon antigenic stimulation	n.d.	+	–
Decrease of caspase activity in $DAPI^{neg}B220^{+}Fas^{hi}PNA^{hi}$ GC B cells	++	+/–	+/–

n.d., not determined (163).

The Individual Contribution of FcγRIIb Deficiency and $Slamf_{129}$ to the Phenotype of the FcγRIIb KO Mouse on Mixed 129/C57BL/6 Background

Independently, in two different laboratories congenic C57BL6 $Slamf_{129}$ mice have been generated. One was generated by intensive backcrossing of the original $Fc\gamma RIIb^{-/-}_{129}$ mouse (161) into C57BL/6 background and selection for offspring in which the *Slamf* locus and the $Fc\gamma RIIb$ KO allele had been segregated (98) resulting in two congenic strains called here as C57BL/6 $Slamf_{129}$ congenic and C57BL/6 $Fc\gamma RIIb^{-/-}_{129}$ $Slamf_{B6}$ congenic, respectively. The other C57BL6 $Slamf_{129}$ congenic mice were generated by a marker-assisted speed congenic approach (163) (**Figure 1**).

The development of autoimmunity was compared between C57BL/6 $Fc\gamma RIIb^{-/-}_{129}$ $Slamf_{B6}$ congenic, C57BL/6 $Slamf_{129}$ congenic and the original $Fc\gamma RIIb^{-/-}_{129}$ mice (98) or between C57BL/6 $Slamf_{129}$ congenic, $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ and the original $Fc\gamma RIIb^{-/-}_{129}$ mice (163). Both C57BL/6 $Fc\gamma RIIb^{-/-}_{129}$ $Slamf_{B6}$ congenic and C57BL/6 $Slamf_{129}$ congenic mice developed very mild disease symptoms whereas the original $Fc\gamma RIIb^{-/-}_{129}$ mice developed severe disease compared to WT C57BL/6 mice. Importantly, the phenotype of the C57BL/6 $Fc\gamma RIIb^{-/-}_{129}$ $Slamf_{B6}$ congenic mouse strain confirmed mainly the phenotype of the $^{Le}Fc\gamma RIIb^{-/-}_{B6}$ mouse [(98); **Table 2**] showing very low ANA titers and little kidney pathology compared to $Fc\gamma RIIb^{-/-}_{129}$ mice.

The development of Spt-GC B cell and T_{FH} responses in C57BL/6 $Slamf_{129}$ congenic, $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ and $Fc\gamma RIIb^{-/-}_{129}$ mice were carefully compared [(163); **Table 3**]. C57Bl/6 $Slamf_{129}$ congenic mice had significantly more GC B cells and T_{FH} and GC T_{FH} cells 12 days after immunization with OVA compared to WT C57BL/6 mice. B cells and DCs from $Slamf_{129}$ congenic mice exhibited stronger antigen presentation in *in vitro* assays compared to B cells and DCs from WT C57BL/6 mice. By using a variety of *in vivo* and *in vitro* assays with naïve B cells it was found that B cell-intrinsic deficiency of FcγRIIb and expression of $Slamf_{129}$ has no effect on proliferation but promotes differentiation of naïve B cells into GC B cells as indicated by increased expression of Aicda and GL-7. The percentage of apoptotic GC B cells was significantly lower in $Fc\gamma RIIb^{-/-}_{129}$ mice compared to WT C57BL/6 mice whereas in C57BL/6 $Slamf_{129}$ congenic and $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ mice this decrease was not significant. This suggests that FcγRIIb deficiency and $Slamf_{129}$ act synergistically to increase the survival of GC B cells in $Fc\gamma RIIb^{-/-}_{129}$ mice. Naïve and activated B cells from $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ and to a lower extent from C57BL/6 $Slamf_{129}$ congenic mice showed an enhanced metabolic capacity compared to B cells from C57BL/6 mice. This enhancement was stronger in $Fc\gamma RIIb^{-/-}_{129}$ mice.

Taken together these observations suggest that $Slamf_{129}$ plays a predominant, and FcγRIIb deficiency a modest role in modulating the Spt-GC B cell and T_{FH} responses. Some of their functions are synergistic others mutually exclusive. GC T_{FH} cell responses are mainly affected by $Slamf_{129}$ [(163); **Table 3**]. By using the experimental model of the V_H chain knockin strain HKIR mentioned earlier (179) it was demonstrated that B cell-specific expression of $Slamf_{129}$ is necessary for the autoreactive B cells to expand in the GC confirming previous observations in C57BL/6 *Sle1* congenic mice (129).

The increased Spt-GC responses in $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ and C57BL/6 $Slamf_{129}$ congenic mice were associated with the production of autoantibodies. However, the titers were much lower than in $Fc\gamma RIIb^{-/-}_{129}$ mice which had also the strongest increase in Spt-GC responses. C57BL/6 $Slamf_{129}$ congenic mice developed higher ANA titers than $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ mice, staining both cytoplasm and nucleus of Hep-2 cells, whereas sera from $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ mice show only cytoplasmic staining patterns (163) confirming previous results with the C57BL/6 $Fc\gamma RIIb^{-/-}_{129}$ $Slamf_{B6}$ congenic mouse strain (98). IgG2b and IgG2c ANA

were significantly increased in C57BL/6 $Slamf_{129}$ congenic mice whereas only IgG2c ANA were significantly increased in $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ mice. With an autoantigen array, it was shown that $Fc\gamma RIIb^{-/-}_{129}$ mice develop high titers of IgG antibodies against a large variety of autoantigens. Several of these antibodies were also present in the serum of $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ mice but their titers were much lower than in $Fc\gamma RIIb^{-/-}_{129}$ mice (163). Unfortunately, sera from C57BL/6 $Slamf_{129}$ congenic mice were not tested in the autoantigen array.

Kidney pathology was absent (98) or very mild, with higher complement deposition than $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ mice (163), in C57BL/6 $Slamf_{129}$ congenic mice, mild in $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ or C57BL/6 $Fc\gamma RIIb^{-/-}_{129}$ $Slamf_{B6}$ congenic mice with higher IgG deposition than in C57BL/6 $Slamf_{129}$ congenic mice, and severe, with highest C3 and IgG deposition compared to the other genotypes, in $Fc\gamma RIIb^{-/-}_{129}$ mice (98, 163). In conclusion, the deficiency of FcγRIIb together with the presence of $Slamf_{129}$ results in a phenotype of the $Fc\gamma RIIb^{-/-}_{129}$ mouse with increased Spt-GC B cell responses characterized by an increase of the following parameters: metabolic activity in B cells, differentiation of B cells into a GC B cell phenotype and GC B cell survival. This is associated with loss of immune tolerance resulting in ANA production and the development of severe lupus-like disease (163). However, the underlying cellular and molecular mechanisms of these associations are not well-understood and the subject of speculation and debate with respect to the role of FcγRIIb in GC (185). This can be illustrated with the surprising observation in the $Fc\gamma RIIb_{NZB}$ KI mouse model mentioned earlier, in which FcγRIIb failed to be upregulated on activated and GC B cells resulting in enhanced early GC responses (11). Upon immunization, these KI mice showed an early and sustained increased affinity maturation of Ag-specific GC B cells. Previous models suggest that low expression of FcγRIIb reduces the BCR activation threshold resulting in less affinity maturation. However, an alternative explanation might be that low FcγRIIb expression increases the survival of bystander Ag non-specific GC B cells and, as a consequence, increases competition for T_{FH} help between Ag-specific and non-antigen specific B cells, resulting in increased affinity maturation (11).

Cell-Type-Specific *FcγRIIb* KO Mouse Models

To determine on what B cell subset(s) and on what myeloid cells FcγRIIb might be involved in a checkpoint for immune tolerance, cell-type-specific $Fc\gamma RIIb^{-/-}$ mice were generated, independently, in two different laboratories. Both the $^{Le}Fc\gamma RIIb^{-/-}_{B6}$ and $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ mouse models, on a pure C57BL/6 background, were originally generated as floxed $Fc\gamma RIIb$ mice ($Fc\gamma RIIb^{fl/fl}_{B6}$) and subsequently crossed with a Cre deleter transgenic mouse to generate the germline $Fc\gamma RIIb^{-/-}_{B6}$ mice discussed earlier. In addition, the $Fc\gamma RIIb^{fl/fl}_{B6}$ mice were also crossed with a variety of cell type-specific Cre transgenic mice (**Table 4**) to generate cell-type-specific $Fc\gamma RIIb^{-/-}_{B6}$ strains that were analyzed in the following models of diseases for which germline $Fc\gamma RIIb$ KOs are highly susceptible: (a) the induced autoimmune diseases CIA, both on permissive (immunization with chicken collagen type II) and non-permissive (immunization with bovine collagen type II) background and (b) anti-glomerular basement membrane antibody (anti-GBM) disease, (c) the spontaneous autoimmune disease lupus-like disease and (d) the non-autoimmune disease antibody-induced NTN.

Deletion of $Fc\gamma RIIb$ in all B cells of the $^{Le}Fc\gamma RIIb^{fl/fl}_{B6}$ mouse by *CD19Cre* did not increase the susceptibility of this mouse for any of the mentioned disease models. Moreover, deletion of $Fc\gamma RIIb$ on a subset of monocytes (*LysMCre*) had no effect on susceptibility for anti-GBM disease. Therefore, it was concluded that FcγRIIb deficiency on B cells or a subset of myeloid cells alone is not sufficient to increase susceptibility to anti-GBM (186). Only pan-myeloid deletion (*cEBPαCre*) of FcγRIIb increased the susceptibility of $^{Le}Fc\gamma RIIb^{fl/fl}_{B6}$ mice for CIA on the permissive background (187) and for the non-autoimmune disease NTN (89). These results suggest that for the protection against induced auto-Ab driven diseases, such as CIA, the role of FcγRIIb on B cells, as a checkpoint for immune tolerance, is less important than its role on myeloid effector cells, controlling downstream antibody effector mechanisms (187). However, it cannot be excluded that in the CIA model FcγRIIb on myeloid cells also plays a role in controlling the afferent phase of the disease, as was recently shown in Yaa^{+} $^{Le}Fc\gamma RIIb^{-/-}_{B6}$ mice that will be discussed later (188).

In contrast to the results with $^{Le}Fc\gamma IIb^{fl/fl}_{B6}$ mice, deletion of FcγRIIb in all B cells (*Mb1Cre*) or in GC and post GC B cells (*Cg1Cre*) in $^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ mice resulted in increased susceptibility for CIA on the non-permissive background and permissive background, respectively. Moreover, susceptibility to CIA was also increased in DC-specific *CD11cCre*/$^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ mice indicating that FcγRIIb is involved in distinct immune tolerance controlling mechanisms (110). The reason for the discrepancy between the phenotypes of the B cell- and DC-specific $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ and $^{Le}Fc\gamma IIb^{-/-}_{B6}$ mice is not known but, given the weak phenotype of the germline $^{Le}Fc\gamma RIIb^{-/-}_{B6}$ mouse, most likely the phenotype of a single cell-type-specific $^{Le}Fc\gamma RIIb^{-/-}_{B6}$ mouse is too weak to be detected with a small cohort of mice. Another partial explanation might be that the B-cell-specific Cre lines used are different. In addition, GC and post GC B cell (*Cg1Cre*) specific $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ mice developed spontaneously ANA, similar to ANA in germline $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ mice, whereas a deficiency in other cell types has no effect. This confirms previous results with transplantation of bone marrow from $Fc\gamma RIIb^{-/-}_{129}$ mice that the role of FcγRIIb in the spontaneous development of ANA is B cell-specific (112) and suggests that FcγRIIb on GC or post GC B cells is a checkpoint for the maintenance of immune tolerance (110) (**Table 4**).

Upon immunization with the NP-CGG model antigen $^{NY}Fc\gamma RIIb^{-}_{B6}{}^{/-}$ and *Mb1Cre*/$^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ mice developed similar increased primary IgG NP-specific Ab responses compared to $^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ mice and all other cell type-specific

TABLE 4 | Disease susceptibility of cell-type-specific *FcγRIIb* KO mice.

Disease \ Mouse strain	*CD19Cre: All B cells*[a,b,c]	*LysMCre: Subset monocytes*[a,d]	*cEBPαCre: pan-myeloid*[b,c]	*CD11cCre: DCs*[c,d]	*Mb1Cre: All B cells*[d]	*Cg1Cre: GC and post GC B cells*[d]
Non-permissive bCIA[c,d]	No increase	No increase $^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ [d]	n.d.	Increase $^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ [d]	Increase $^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ [d]	No increase $^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ [d]
Permissive cCIA[c,d]	No increase $^{Le}Fc\gamma RIIb^{fl/fl}_{B6}$ [c]	n.d.	Increase $^{Le}Fc\gamma RIIb^{fl/fl}_{B6}$ [c]	No increase $^{Le}Fc\gamma RIIb^{fl/fl}_{B6}$ [c]	n.d.	Increase similar to $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ [d]
KRN arthritis[d]	n.d.	Increase $^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ [d]	n.d.	n.d.	n.d.	n.d.
Anti-GBM disease[a]	No increase $^{Le}Fc\gamma RIIb^{fl/fl}_{B6}$ [a]	No increase $^{Le}Fc\gamma RIIb^{fl/fl}_{B6}$ [a]	n.d.	n.d.	n.d.	n.d.
Lupus-like disease[d]	n.d.	No ANA $^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ [d]	n.d.	No ANA $^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ [d]	No ANA $^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ [d]	ANA similar to $^{NY}Fc\gamma RIIb^{-/-}_{B6}$ [d]
NTN[b]	No increase $^{Le}Fc\gamma RIIb^{fl/fl}_{B6}$ [b]	n.d.	Increase $^{Le}Fc\gamma RIIb^{fl/fl}_{B6}$ [b]	n.d.	n.d.	n.d.
Immunization[d]	n.d.	No increase in IgG response $^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ [d]	n.d.	No increase in IgG response $^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ [d]	Increased primary/secondary IgG response $^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ [d]	Increased secondary IgG response $^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ [d]

Germline FcγRIIb KO mice showed increased susceptibility to all diseases listed in the table compared with C57BL/6 mice.
[a] *Sharp et al. (186).*
[b] *Sharp et al. (89).*
[c] *Yilmaz-Elis et al. (187).*
[d] *Li et al. (110).*
n.d., not determined.

$^{NY}Fc\gamma RIIb^{-/-}_{B6}$ mice. In contrast, secondary IgG Ab responses were increased in both *Mb1Cre/*$^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ and *Cg1Cre/*$^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ mice compared with $^{NY}Fc\gamma RIIb^{fl/fl}_{B6}$ mice. This suggests that FcγRIIb is a B cell-intrinsic negative regulator of both primary and secondary IgG responses (110).

Although individually not sufficient to induce substantial autoimmunity, epistasis between the *Yaa* locus, the $^{Le}Fc\gamma RIIb^{-/-}_{B6}$ alleles and the C57BL/6 genome results in severe lupus-like disease (109) (**Figure 1**). The cell-type-specific role of FcγRIIb in this genetic disease model was studied (188). The *Yaa*$^{+}$*/CD19Cre/*$^{Le}Fc\gamma RIIb^{fl/fl}_{B6}$ mice developed milder lupus-like disease than *Yaa*$^{+}$/$^{Le}Fc\gamma RIIb^{-/-}_{B6}$ mice similar to the disease in *Yaa*$^{+}$*/C/EBPα Cre/*$^{Le}Fc\gamma RIIb^{fl/fl}_{B6}$ mice whereas *Yaa*$^{+}$*/CD11cCre/*$^{Le}Fc\gamma RIIb^{fl/fl}_{B6}$ mice stayed disease free, like *Yaa*$^{+}$/$^{Le}Fc\gamma RIIb^{fl/fl}_{B6}$ mice. This suggests that besides on B cells FcγRIIb on myeloid cells, but surprisingly not on DCs, contributes to the protection against spontaneous loss of immune tolerance in this mouse model. This confirms the observation with CIA in mice (110), discussed earlier, that FcγRIIb can be involved in different immune tolerance controlling mechanisms.

Strikingly, in the two strains with FcγRIIb deficient myeloid cells (*Yaa*$^{+}$/$^{Le}Fc\gamma RIIb^{-/-}_{B6}$ and *Yaa*$^{+}$*/C/EBPα Cre/*$^{Le}Fc\gamma RIIb^{fl/fl}_{B6}$) but not in the strain with B cell-specific FcγRIIb deficiency (*Yaa*$^{+}$*/CD19Cre/*$^{Le}Fc\gamma RIIb^{fl/fl}_{B6}$) the frequency of peripheral Ly6C^{-}, but not Ly6C^{+} monocytes was increased. Monocytosis, an FcRγ dependent expansion of the monocyte compartment consisting mainly of Ly6C^{-} monocytes, is associated with the development of lupus nephritis in *Yaa*$^{+}$ lupus-prone mice. It has been reported that Ly6C^{+} monocytes mature in the circulation and are the precursors for Ly6C^{-} monocytes (189). Deficiency of FcγRIIb most likely accelerates the maturation of monocytes in *Yaa*$^{+}$/$^{Le}Fc\gamma RIIb^{-/-}_{B6}$ mice. Compared to Ly6C^{+} monocytes, mature Ly6C^{-} monocytes express significantly higher B cell-stimulating cytokines such as BSF-3, IL-10, and IL-1β, DC markers including CD11c, CD83, Adamdec1, and the anti-apoptotic factors Bcl2 and Bcl6. This makes monocytes the most promising FcγRIIb expressing candidate myeloid cells to modulate B cell tolerance (188, 190). The transcriptome of Ly6C^{-} monocytes suggests that they are long-lived and committed to developing into DCs.

Whether this monocyte-dependent tolerance breaking mechanism is unique for *Yaa*$^{+}$/$Fc\gamma RIIb^{-/-}_{B6}$ mice is not known but it is striking that also in SLE patients the serum levels of anti-dsDNA Abs highly correlate with the percentage of non-classical monocytes (191). Like mouse Ly6C^{-} monocytes, the human counterpart CD14^{low}CD16^{+} monocytes secrete high amounts of IL-1β in a TLR7-TLR8-MyD88–dependent manner (192).

CONCLUDING REMARKS

Forward and reverse genetics have provided convincing evidence that FcγRIIb is an important autoimmune susceptibility gene, involved in the maintenance of peripheral tolerance both in human and mice. In humans, a number of GWAS studies showed an association between a SNP *(rs1050501)* in the *FCGR2B* gene, causing a missense mutation (*FCGR2B*^{T232}) resulting in impaired FCGR2B function, and susceptibility to SLE. Meta-analyses confirmed that *FCGR2B*^{T232} homozygosity is one of the

strongest associations in SLE. Association of *rs1050501*with RA has also been reported.

In mice, the situation is more diffuse. Analysis of a variety of C57BL/6 mice congenic for the NZW and NZB haplotypes of *Fcγ RIIb*, with decreased expression, did not reveal clear unambiguous results with respect to the contribution of these haplotypes to the autoimmune phenotypes of these mice. The mechanism by which natural FcγRIIb variants contribute to autoimmunity is not well-understood.

The first $Fc\gamma RIIb^{-/-}$ mouse, generated by gene targeting in 129 derived ES cells and backcrossed into C57BL/6 background ($Fc\gamma RIIb^{-/-}_{129}$ mice), exhibited a surprisingly strong spontaneous autoimmune phenotype suggesting that FcγRIIb deficiency initiates loss of immune tolerance. However, independent studies with $Fc\gamma RIIb^{-/-}_{129}$ autoimmune V_H chain knockin mice pointed to a central role of FcγRIIb in a late immune tolerance checkpoint, that prevents autoimmunity by suppressing the production of autoreactive IgG from B cells, that escape negative selection in the GC and enter the AFC pathway. This should mean that FcγRIIb deficiency is mainly an amplifier of autoimmunity caused by other autoimmune susceptibility loci, rather than a primary initiator of the loss of immune tolerance. That was confirmed by the observation that $Fc\gamma RIIb^{-/-}$ mice on a pure C57BL/6 background ($Fc\gamma RIIb^{-/-}_{B6}$) have a much milder autoimmune phenotype than $Fc\gamma RIIb^{-/-}_{129}$ mice but when backcrossed into a mouse strain carrying the autoimmune susceptibility *Yaa* locus succumb to lupus-like disease. The strong autoimmune phenotype of the $Fc\gamma RIIb^{-/-}_{129}$ mouse could be explained by epistatic interactions between the C57BL/6 genome, the FcγRIIb KO allele and the 129 derived sequences (*Sle16*) flanking the *Fcγ RIIb* KO allele, containing the autoimmunity associated $Slamf_{129}$ (haplotype 2) gene cluster.

Spt-GC B and T_{FH} cells are activated, modestly (mainly B cells) in $Fc\gamma RIIb^{-/-}_{B6}$ mice, moderately in C57BL/6 $Slamf_{129}$ congenic mice and strongly in $Fc\gamma RIIb^{-/-}_{129}$ mice compared to Spt-GC B and T_{FH} cells in WT C57BL/6 mice. This was associated with a corresponding increase in ANA production, suggesting that FcγRIIb deficiency, besides enhancing autoimmunity caused by other autoimmune susceptibility loci, might play a modest role in the induction of the loss of immune tolerance in the GC, explaining the development with low penetrance of low ANA titers in $Fc\gamma RIIb^{-/-}_{B6}$ mice. An alternative explanation is that the low ANA titers in $Fc\gamma RIIb^{-/-}_{B6}$ mice reflect the natural background of autoreactive B cells in the GC that are prevented to enter the AFC pathway in the presence of FcγRIIb. The analysis of the development of self-reactive GC B cells and plasma cells by large scale Ig cloning from single isolated B cells, as performed with $Fc\gamma RIIb^{-/-}_{129}$ mice, should be repeated in $Fc\gamma RIIb^{-/-}_{B6}$ mice, to determine how FcγRIIb deficiency influences the frequency at which autoreactive ANA-expressing B cells participate in GC reactions, and develop in plasma cells, under physiological conditions, without the confounding effect of $Slamf_{129}$ expression.

Studies with cell-type-specific FcγRIIb deficient mice revealed that besides on B cells, FcγRIIb on DCs and monocytes can also contribute to the maintenance of immune tolerance, indicating that FcγRIIb is involved in different immune tolerance maintaining mechanisms. Series of observations suggest that on B cells impaired FcγRIIb function effects not only antibody titers but also affinity maturation and memory responses of B cells and plasma cell homeostasis associated with an increase in the production of autoantibodies. However, the underlying cellular and molecular mechanisms are not well-understood. Most likely new model systems including adoptive cell transfer and tools such as cell type-specific KO mice, to study the GC reaction, are required to answer these questions.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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