Published in final edited form as:
Cell Immunol. 2010 ; 266(1): 24–31. doi:10.1016/j.cellimm.2010.08.007.

Defining the immunological phenotype of Fc receptor-like B (FCRLB) deficient mice: confounding role of the inhibitory FcγRIIb

Keiji Masuda[1], **Hiromi Mori**[1], **Osamu Ohara**[2,3], **Manabu Nakayama**[3], **Ji-Yang Wang**[1], and **Peter Burrows**[4,5,*]

[1]Laboratory for Immune Diversity, Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Yokohama 230-0045, Japan

[2]Laboratory for Immunogenomics, Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Yokohama 230-0045, Japan

[3]Department of Human Genome Research, Kazusa DNA Research Institute, Kisarazu, Chiba 292-0818, Japan

[4]Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

[5]Department of Genetics, University of Alabama at Birmingham, Birmingham, AL 35294, USA

Abstract

Fc receptor-like A (FCRLA) and FCRLB have homology to the transmembrane FCRL family members (FCRL1–6) and to the conventional receptors for the Fc portion of immunoglobulin, but uniquely are cytosolic proteins expressed in B cells. Here we describe the phenotype of *Fcrlb* gene targeted mice. B cell development and *in vitro* responses are normal; however, antibody responses to a T-dependent antigen are elevated. The gene encoding the inhibitory FcγRIIb is located nearby *Fcrlb*. Although *Fcrlb* gene targeting had no effect on the function or basal expression of FcγRIIb, its expression was reduced following activation. This abnormal regulation was due to co-inheritance of *Fcgr2b* and the mutant *Fcrlb* allele from the 129 ES cells. A promoter polymorphism in the 129/Sv *Fcgr2b* allele results in diminished upregulation of FcγRIIb following B cell activation. Thus, we speculate that the enhanced antibody response seen in the FCRLB-deficient mice may be due to the *Fcgr2b* promoter.

Keywords

Fc receptor; Gene targeting; FcγRIIb; Antibody response; Germinal center

[*]**Corresponding author:** Peter D. Burrows, UAB 406 SHEL, 1530 Third Avenue South, Birmingham, AL USA 35294-2182, peterb@uab.edu, 205.934.6529, FAX 205.996.9917.

INTRODUCTION

Receptors for the constant region of immunoglobulins (FcR), FcγRI, FcγRII, FcγRIII, FcεRI, are found on cells of both myeloid and lymphoid lineages [4;12;32]. Members of this "classical" FcR family are known to have important regulatory roles in both cell mediated and humoral immunity, including feedback suppression of B cell responses, regulation of hypersensitivity reactions, and the induction of cellular cytotoxicity. Other FcRs include the recently identified FcμR, which binds IgM [14;36], Fcα/μR [11;13;34], which binds IgA and IgM, the polymeric immunoglobulin receptor (polyIgR) [29] that mediates transcytosis of IgA across mucosal epithelial surfaces, the neonatal FcRn [15], a receptor related to MHC class I that mediates perinatal transfer of Ig and maintenance of basal immunoglobulin levels in adults, and the FcαR (CD89) [26], a receptor for IgA that is present on myeloid cells in humans and rats, but curiously not in mice [20]. The *FcRn* gene is located on chromosome 19q13.3 [10] and *CD89* is found in the leukocyte receptor complex on chromosome 19q13.4 [17;38;39], whereas the genes encoding the other Fc receptors are located on human chromosome 1q32

There has been an unexpected recent harvest of FcR related genes from the human chromosome 1q region. Six human Fc receptor-like (*FCRL 1–6*) genes have been identified. They encode type I transmembrane proteins with similar extracellular Ig-like domains and cytoplasmic regions that contain consensus tyrosine based motifs, suggesting an inhibitory (ITIM) or activating (ITAM) signaling function for these receptors [5]. Until the very recent identification of MHC class II as a ligand for FCRL6 [33], the ligands for FCRL 1–6 have been unknown, but none of these receptors has been convincingly shown to bind immunoglobulins. During the characterization of these extended FcR family members, we identified two unusual relatives, *FcRX* and *FcRY*, in the human 1q region [6;21]. *FcRX* is also termed *FREB* (Fc receptor homolog expressed in B cells) and *FcRL* (FcR-like) because of its independent identification by other laboratories [7;25]. Similarly, *FcRY* is called *FcRL2* and *FREB2* [3;40]. The HUGO Gene Nomenclature Committee has recently adopted *FCRLA* and *FCRLB* as the approved human symbols for these genes; the mouse genes are designated *Fcrla* and *Fcrlb* [19].

Both FCRLA and FCRLB proteins have unusual features that distinguish them from other members of the FCRL family. Most notably, they are intracellular proteins rather than transmembrane receptors [3;7;25;40]. The only available information about the expression of these receptors at the protein level comes from studies in humans. Among hemopoietic cells FCRLA is expressed only in B cells, with the highest levels found in the germinal center B cells. Wilson and Colonna found that FCRLB expressing cells are also present in the germinal centers of tonsils [40]. However, the $FCRLB^+$ cells were extremely rare, in tissue sections many germinal centers contained no $FCRLB^+$ cells, and were non-proliferating. This is in striking contrast to $FCRLA^+$ cells, which are abundant and enriched among proliferating germinal center centroblasts [7]. Moreover, FCRLA and FCRLB were not co-expressed in the same cells. Due to the lack of suitable mAb and the low levels of mRNA, FCRLB expression in mice has only been analyzed by RT-PCR. We found that *Fcrlb* transcripts could be detected in all B cell subsets in the spleen, although they were somewhat reduced in germinal center B cells, in keeping with our observation that *Fcrlb* expression is highest in non-proliferating cells [21]. By contrast, Wilson and Colonna found expression restricted to germinal center B cells and an undefined population of cells expressing B220, CD21, and CD23 [40]. The basis for this discrepancy in the *Fcrlb* expression profile is unclear, but may be due to the markers used for GC B cell isolation, peanut agglutinin versus the monoclonal antibody GL7.

Given the difficulty in analyzing FCRLB expression *in vivo* and *in vitro*, we reasoned that its function might be best elucidated by a genetic approach. Our analysis of *Fcrlb* gene targeted mice is described here.

METHODS

Generation of Fcrlb knockout mice

To isolate the genomic fragment containing the *Fcrlb* gene, we screened a BAC clone library of 129-derived R1 ES cells with a primer set (FcRY/s20086: 5'-TCAGGGAAGAGGTTATCAGG-3'; FcRY/as20404: 5'-CAACCCAACTCAAGAAATCC-3'). The isolated BAC clone was confirmed to contain the *Fcrlb* gene by sequencing the 5' and 3' end of the insert, as well as by digestion with multiple restriction enzymes. A 5.6-kb *Bgl*II fragment and a 3.2-kb *Sac*I-*Not*I fragment were used as the 5' and 3' homology regions of the targeting vector, respectively, to replace exons 1, 2 and a portion of 3 along with a ~2-kb promoter region with the neomycin gene. 129-derived R1 ES cells were transfected with the linearized targeting vector and 2 days after transfection cultured in the presence of 600 μg/ml G418 and 2 μM of GANC (only for the first two days) as described previously [22]. Homologous recombination was verified by long range genomic PCR using primers flanking 5' and 3' homology regions, and neo primers. (See Fig S1B legend for details.) Chimeric mice were bred with C57BL/6 mice to obtain heterozygotes, which were then crossed to obtain homozygotes. Mouse genotypes were identified by genomic PCR using primers FcRY/s8055 (5'-TGGCTTCTCTTTAGTGATGC-3'), FcRY/as8642 (5'-ATGTGGTTGCTGGGACTTGA-3') and neo/s at 95 °C for 2 min followed by 30 cycles of amplification at 95 °C for 10 s, 60 °C for 10 s and 72 °C for 90 s. The WT and KO allele give rise to a 590-bp and 880-bp band, respectively. *Fcrlb* mRNA expression was analyzed by PCR using primers s144 (5'-CAGGCAGAGTCATTATGTGG-3'), as561 (5'-GCCGTCGTGGTAGTAGTGAA-3') and FW169 (5'-TTAGCACTCTCTGGTACCTGG-3') at 95 °C for 2 min followed by 35 cycles of amplification at 95 °C for 15 s, 55 °C for 10 s and 72 °C for 2 min. Mice were housed in specific pathogen free conditions and all experiments were approved by the Animal Facility Committee of the RIKEN Yokohama Institute (Permission no. 20-025).

Flow cytometry analysis and proliferation assays

Flow cytometry analysis was performed as described previously [23]. For proliferation, purified spleen B cells were seeded in a 96 well plate at 5×10^5/ml, 100 μl/well, and stimulated for 2 days with F(ab')2 or whole anti-IgM with or without soluble CD40 ligand. ^{3}H-thymidine (1 μCi/well) was added during the last 8 h and thymidine uptake was measured as described previously. [23].

Immune response and ELISPOT assay

Immune responses were tested essentially as previously described [24]. Briefly, four WT and five *Fcrlb*-KO mice (8 wk-old) were injected i.p. with 100 μg of NP-CGG (Biosearch Technologies, Novato, CA) precipitated with alum. The mice were boosted with the same antigen at age 15 wks. Mice were bled weekly and serum titers of NP-specific IgG1 were analyzed by ELISA, using NP-specific monoclonal high (clone C6) and low (clone N1G9) affinity antibodies as standards.

The ELISPOT assay was performed as described [24]. Briefly, Multiscreen HTS filter plate (Millipore) were coated with 50 μg/ml of NP3-BSA or NP30-BSA at 4°C overnight. The coated plate was then washed with PBS-T (PBS containing 0.1% Tween 20) 3 times and blocked with PBS containing 1% BSA for 1 h at RT. Splenocytes (5×10^5, $2.5\times10^5$and

1.25×10^5) were then seeded and incubated at 37°C for 100 min in a CO_2 incubator. The plate was then washed twice with PBS-T containing 50 mM EDTA, 3 times with PBS-T and then blocked again with PBS containing 1% BSA for 1 h at RT. The plate was further incubated with alkaline phosphatase-conjugated goat anti-mouse IgG1 antibodies (1 μg/ml in PBS containing 1% BSA) at 37°C for 60 min in a CO_2 incubator, washed 4 times with PBS-T and developed with BCIP/NBT reagent (MOSS INC) for 2–3 min. The plate was then washed 4 times with H_2O, air dried and colonies were counted using an IMMUNOSPOT Analyzer (CTL Analyzers LLC, Cleveland, OH). The number of colonies obtained with different number of splenocytes was converted to number of colonies/10^5 cells and the average numbers are shown.

Expression of FcγRIIb

Spleen B cells were purified using IMAG negative sorting (BD) and then cultured for 2 days in a 24 well plate at 5×10^5 cells/ml, 1ml/well in the presence of 20 μg/ml of LPS or 10 μg F(ab')2 anti-IgM plus 20 ng/ml of IL4. The cells were then stained with FITC-conjugated anti-FcγRIIb (2.4G2, Rat IgG2b, BD) plus APC-conjugated B220 and the FcγRIIb expression was analyzed on gated $B220^+$ cells. As a control, the same cultured cells were stained with FITC-conjugated control rat IgG2b and APC-B220. 98% of the cultured cells were $B200^+$ both in WT and KO mice.

Analysis of the polymorphism in the FcgRIIb promoter

129 mice are known to have a polymorphism in the *Fcgr2b* gene promoter, i.e., a 16-bp deletion when compared to C57BL/6 mice. We analyzed this deletion by genomic PCR using primers Fcgr2b/s (5'-GTTGATCTTCATTTTACAGAC-3') and Fcgr2b/as (5'-TCTGTGCCCTAGTCCTGAATC-3') at 95 °C for 3 min followed by 35 cycles of amplification at 95 °C for 5 s, 55 °C for 10 s and 72 °C for 30 s. The B6- and 129-derived genomes give rise to 164-bp and 148-bp PCR products, respectively, that were resolved on 2% agarose gels.

RESULTS

Generation of *FCRLB*-deficient mice

The *Fcrlb* targeting vector (Fig 1A and Supplemental Fig. 1A) was designed to replace ~1.5 kb of the 5' flanking region of the *Fcrlb* gene as well as part of the coding region (exons 1, 2 and the 5' end of exon 3) with a *neo* gene in the opposite transcriptional orientation. Homologous recombination in the R1 ES cells (129/Sv) containing the targeted allele was confirmed by Southern blots and genomic PCR (Fig S1). The ES clones were introduced into C57BL/6 blastocysts by an aggregation method and the resultant male chimeric mice were mated with C57BL/6 females to generate heterozygous offspring. These were intercrossed to obtain homozygous *Fcrlb*-gene targeted mice. Initial characterization of the mice was performed when they had been backcrossed to C57BL/6 mice for two generations. Detailed analysis of NP-antibody responses, B cell proliferative responses, and FcγRIIb expression was performed on mice backcrossed for eight generations.

Lacking an antibody able to detect FCRLB expression, we performed RT-PCR to identify *Fcrlb* mRNA in purified splenic B cells from wild type and knockout mice to confirm the successful knockout of the *Fcrlb* gene. Using a sense primer (S144) located in exon 1 and an anti-sense primer (as561) located in exon 4 of the *Fcrlb* gene, a major band of the expected size (~400 bp) was amplified from RNA samples derived from wildtype B cells (Fig. 1B, WT lane 1). Several larger bands were also detected, most likely derived from incompletely spliced transcripts. No bands were observed when this same primer pair was used to amplify RNA from the knockout B cells (Fig. 1B, KO lane 1), as expected since exon 1 is within the

region deleted by the targeting construct. When the upstream primer was replaced with a primer (FW169) in exon 3, which is retained in the mutant allele, a major band of ~250 bp was amplified from B cell RNA of both wildtype and knockout mice (Fig. 1B, lanes 2). The RNA species from the knockout B cells did not contain the *neo* sequence (Fig. 1B KO lane 3) or the loxP sequence (not shown) present in the targeting vector. Since the native promoter was deleted by the targeting vector, this RNA species is likely derived from cryptic transcription initiation within the remnant *Fcrlb* gene, or by splicing of an upstream sequence onto exon 3 of the gene. We attempted to define the origin of this transcript using 5' RACE (rapid amplification of cDNA ends) but our repeated attempts were unsuccessful.

Phenotype of the FCRLB-deficient mice

We first performed a detailed analysis of the basal immune phenotype of the *Fcrlb* knockout mice. There were no significant differences in the levels of serum IgM, IgG subclasses, or IgA in 10–12 week old wildtype and knockout mice (Fig. S2). B cell development in the bone marrow, from the proB to the mature B cell stage was unaffected by the absence of FCRLB, and in the spleen, the percentage and absolute numbers of total B cells and B cell subsets (immature, transitional, mature, follicular and marginal zone) were normal (Representative flow cytometry data, Fig. S3A, B; Summary data, Fig. 2.) Although T cells do not express FCRLB, we examined this lineage in the event that FCRLB deficiency had any unanticipated indirect effect. T cell development in the thymus was normal, as was the frequency of CD4 and CD8 T cells in the spleen (Fig S3C).

B cell function in the FCRLB-deficient mice was first tested *in vitro*. Purified splenic B cells were stimulated with the BCR agonist anti-IgM, the TLR4 agonist LPS, and a surrogate for T cell help, CD40L, alone or in combination (Fig. 3). No significant differences in the responses of the knockout and wildtype B cells to any of these stimuli were observed.

The *in vivo* function of the knockout B cells was tested after the mice had been backcrossed for eight generations. We examined the T-dependent response in detail, both in terms of primary and secondary responses and in terms of the affinity of the antibodies produced. Since FCRLB may be expressed in murine GC B cells, we considered that its absence might influence these parameters. The primary NP response in *Fcrlb* knockout mice was somewhat elevated compared to that in wildtype mice equivalent, and the secondary response was even more enhanced (Fig. 4). This increase was apparent in the total anti-NP IgG1 antibody levels and was even more striking in the high affinity anti-NP antibodies.

We next determined whether this increase in serum antibody titers was due to an increase in the numbers of antibody secreting cells or in the amount of antibody produced per cell. An ELISPOT assay was performed six weeks after the secondary immunization with $NP_{16}CGG$, and the numbers of both total and high affinity AFC were increased in spleen and bone marrow of the FCRLB-deficient mice (Fig. 5).

Does the inhibitory FcγRIIb have a role in the phenotype of the Fcrlb knockout mice?

The *Fcgr2b* gene, which is ~37.5 kb telomeric from the *Fcrlb* gene, encodes an ITIM-bearing receptor that can potently inhibit B cell responses. This inhibition occurs when IgG antigen-antibody complexes crosslink the BCR with the inhibitory FcγRIIb, which recruits SHIP1 to its ITIM motif and ultimately dampens BCR signaling by preventing intracellular calcium mobilization [1]. From the outset we recognized the possibility that our targeting of the *Fcrlb* gene might influence expression of the *Fcgr2b* gene in *cis*, and that this could have profound effects on B cell responses. This concern was obviated, however, by our initial analysis. A classical assay to demonstrate the inhibitory effect of FcγRIIb is to compare proliferation of B cells stimulated with intact anti-IgM antibodies, which can colligate the

BCR and FcγRIIb, to cells stimulated with F(ab')2 antibodies, which cannot. The proliferation of both wild type and knockout B cells was reduced approximately sevenfold when intact anti-IgM antibodies were used as the stimulus (Fig. 6). Co-stimulation with CD40L can partially rescue this FcγRIIb-mediated inhibition, and again, the response of wildtype and knockout B cells was identical. These results suggested that the *in vitro* function of FcγRIIb on B cells was not influenced by targeting the *Fcrlb* gene. Further evidence in support of this idea was obtained by flow cytometry. The levels of FcγRIIb detected by the 2.4G2 mAb were the same, or if anything slightly higher, on the knockout resting B cells compared to the wildtype B cells (Fig. 7). Thus the basal expression of FcγRIIb was unaffected by our gene targeting strategy. Interestingly, however, its expression was significantly dysregulated following B cell activation. Compared to wildtype, the level of FcγRIIb on the knockout B cells was more than 1.5 fold less following LPS stimulation and more than 2 fold less after stimulation with anti-IgM plus IL4.

To further evaluate a possible role for FcγRIIb in the *Fcrlb* knockout phenotype, we tested the mice for autoantibody production. ELISA titers of anti-nuclear antibodies were marginally increased as the mice aged (Fig. 8). A similar marginal increase in ANA titers has been observed in C57BL/6 mice congenic for the 129 strain-derived chromosome 1 interval from 87.9 to 100 cM, a region that contains the *Fcgr2b* gene [2].

In search of a possible explanation for the abnormal regulation of FcγRIIb expression in activated B cells, we focused on the *Fcgr2b* promoter. A polymorphism in this promoter has been identified in certain autoimmune mouse strains and in some cases is thought to promote autoantibody formation due to B cell hyperactivation [8;9;16;30;41]. This same polymorphism is present in the 129 mouse strain, from which the ES cells used for *Fcrlb* targeting were derived. A genomic PCR assay was designed to distinguish 129 and C57BL/6 promoters by size of the amplified fragment and, indeed, the *Fcrlb* gene targeted mice possessed the 129-derived *Fcgr2b* promoter polymorphism (Fig. S4).

DISCUSSION

Our studies here report for the first time on the phenotype of the knockout of one of the intracellular FcRL family members, *Fcrlb*. The mutant mice developed normally and the absence of FCRLB had no obvious effect on the differentiation of B and T lineage cells. The *in vitro* responses of the knockout B cells stimulated with the BCR agonist anti-IgM, the TLR4 agonist LPS, and a surrogate for T cell help, CD40L, as well as various combinations of these stimuli, were also normal. However, the *in vivo* antibody response to a T dependent antigen was increased in the knockout mice.

Fcrlb is related to *Fcrla*, another member of the FcR like gene family. In humans both *FCRLA* and *FCRLB* encode intracellular proteins that are preferentially, although not exclusively expressed in germinal center B cells [6;7;25;40]. The human FCRLA is found in the endoplasmic reticulum where it binds intracellular immunoglobulin and may be involved in retention of the secretory form of antibody in B cells (T. Santiago, L. Hendershot, A. Taranin, and PDB *manuscript submitted*). Human FCRLB is also expressed in the germinal center but only by very few of the B cells [40]. In considering a function for FCLRB, we reasoned that, like FCRLA, it might retain secreted immunoglobulin in B cells. Therefore, in the absence of FCRLB there might be more Ig secreting cells, or more immunoglobulin secreted per cell. Our data showing increased serum antibody responses and an increase in AFC following immunization in the FCRLB-deficient mice were consistent with this hypothesis.

As a computational approach to determining FCRLB function, we sought evidence for SNPs in the human *FCRLB* gene. Several FCRLB SNPs have been described in the coding region, but none in the 5' flanking region, and some of these result in non-synonymous amino acid substitutions. http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=127943. However, there have been no published studies describing immunological characterization of individuals with different FCRLB SNP haplotypes. Surprisingly, though, one of these FCRLB alleles has been nominally associated as a risk factor for cardiovascular disease [18;35], although among 74 such risk factor genes, FCRLB was not in the top ten based on probability analyses. By contrast there is significant association with SLE and an *Fcgr2b* promoter polymorphism [27;28;37]

Targeting the *Fcrlb* gene had no effect on the basal expression levels of FcγRIIb, the inhibitory Fc receptor expressed on B cells and encoded by a neighboring gene. However, expression of FcγRIIb on the knockout B cells was reduced compared to wildtype B cells following activation, consistent with previous studies [30;41]. Further analysis revealed the co-inheritance of the targeted *Fcrlb* and $FcgR2b^{129}$ alleles. The latter contains a promoter polymorphism that results in decreased expression of the receptor on activated B cells [30;31;41]. Given the very limited expression of *Fcrlb* in B cells compared with the powerful effects of FcγRIIb on B cell responses, the elevated antibody response in the FCRLB-deficient mice may be attributable to the presence of this promoter polymorphism. The modest increase in anti-nuclear antibodies in the *Fcrlb* knockout mice harboring the $FcgR2b^{129}$ allele is consistent with this interpretation, since a similar observation was made in C57BL/6 mice congenic for 129-derived chromosome 1 loci in this region [2]. Ultimately, however, these speculations will have to be tested by defining the individual contribution of the *Fcrlb* and *FcgR2b* genes by performing gene targeting of *Fcrlb* in ES cells of C57BL/6 origin. Thus our findings, while not entirely conclusive in terms of FCRLB function, nonetheless confirm the functional consequences of the *FcgR2b* promoter polymorphism on antibody responses. Moreover, investigators interested in using gene targeting to define functions of other members of the FCRL family, notably FCRL1 and FCRL5 which are expressed on B cells, should bear these new findings in mind.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like thank our UAB and RCAI colleagues for helpful discussions during the preparation of this manuscript. These studies were supported in part by an RCAI International Collaboration Award (J-YW and PDB) an NIH NIAID R56 AI069365 (PDB).

References

1. Bolland S, Pearse RN, Kurosaki T, Ravetch JV. SHIP modulates immune receptor responses by regulating membrane association of Btk. Immunity 1998;8:509–516. [PubMed: 9586640]
2. Carlucci F, Cortes-Hernandez J, Fossati-Jimack L, Bygrave AE, Walport MJ, Vyse TJ, Cook HT, Botto M. Genetic dissection of spontaneous autoimmunity driven by 129-derived chromosome 1 Loci when expressed on C57BL/6 mice. J.Immunol 2007;178:2352–2360. [PubMed: 17277141]
3. Chikaev NA, Bykova EA, Najakshin AM, Mechetina LV, Volkova OY, Peklo MM, Shevelev AY, Vlasik TN, Roesch A, Vogt T, Taranin AV. Cloning and characterization of the human FCRL2 gene. Genomics 2005;85:264–272. [PubMed: 15676285]
4. Daeron M. Fc receptor biology. Annu.Rev.Immunol 1997;15:203–234. [PubMed: 9143687]
5. Davis RS. Fc receptor-like molecules. Annu.Rev.Immunol 2007;25:525–560. [PubMed: 17201682]

6. Davis RS, Li H, Chen CC, Wang Y-H, Cooper MD, Burrows PD. Definition of an Fc receptor related gene (*FcRX*) expressed in human and mouse B cells. International Immunology 2002;14:1075–1083. [PubMed: 12202404]
7. Facchetti F, Cella M, Festa S, Fremont DH, Colonna M. An unusual Fc receptor-related protein expressed in human centroblasts. Proc.Natl.Acad.Sci.U.S.A 2002;99:3776–3781. [PubMed: 11891275]
8. Jiang Y, Hirose S, Abe M, Sanokawa-Akakura R, Ohtsuji M, Mi X, Li N, Xiu Y, Zhang D, Shirai J, Hamano Y, Fujii H, Shirai T. Polymorphisms in IgG Fc receptor IIB regulatory regions associated with autoimmune susceptibility. Immunogenetics 2000;51:429–435. [PubMed: 10866109]
9. Jiang Y, Hirose S, Sanokawa-Akakura R, Abe M, Mi X, Li N, Miura Y, Shirai J, Zhang D, Hamano Y, Shirai T. Genetically determined aberrant down-regulation of FcgammaRIIB1 in germinal center B cells associated with hyper-IgG and IgG autoantibodies in murine systemic lupus erythematosus. Int.Immunol 1999;11:1685–1691. [PubMed: 10508186]
10. Kandil E, Egashira M, Miyoshi O, Niikawa N, Ishibashi T, Kasahara M. The human gene encoding the heavy chain of the major histocompatibility complex class I-like Fc receptor (FCGRT) maps to 19q13.3. Cytogenet.Cell Genet 1996;73:97–98. [PubMed: 8646894]
11. Kikuno K, Kang DW, Tahara K, Torii I, Kubagawa HM, Ho KJ, Baudino L, Nishizaki N, Shibuya A, Kubagawa H. Unusual biochemical features and follicular dendritic cell expression of human Fcalpha/mu receptor. Eur.J.Immunol 2007;37:3540–3550. [PubMed: 18000956]
12. Kinet JP. The high-affinity IgE receptor (FcεRI): from physiology to pathology. Annu.Rev.Immunol 1999;17:931–972. [PubMed: 10358778]
13. Kinet JP, Launay P. Fc α/μR: single member or first born in the family? Nat.Immunol 2000;1:371–372. [PubMed: 11062492]
14. Kubagawa H, Oka S, Kubagawa Y, Torii I, Takayama E, Kang DW, Gartland GL, Bertoli LF, Mori H, Takatsu H, Kitamura T, Ohno H, Wang JY. Identity of the elusive IgM Fc receptor (FcmuR) in humans. J.Exp.Med 2009;206:2779–2793. [PubMed: 19858324]
15. Lencer WI, Blumberg RS. A passionate kiss, then run: exocytosis and recycling of IgG by FcRn. Trends Cell Biol 2005;15:5–9. [PubMed: 15653072]
16. Lin Q, Xiu Y, Jiang Y, Tsurui H, Nakamura K, Kodera S, Ohtsuji M, Ohtsuji N, Shiroiwa W, Tsukamoto K, Amano H, Amano E, Kinoshita K, Sudo K, Nishimura H, Izui S, Shirai T, Hirose S. Genetic dissection of the effects of stimulatory and inhibitory IgG Fc receptors on murine lupus. J.Immunol 2006;177:1646–1654. [PubMed: 16849473]
17. Liu WR, Kim J, Nwankwo C, Ashworth LK, Arm JP. Genomic organization of the human leukocyte immunoglobulin-like receptors within the leukocyte receptor complex on chromosome 19q13.4. Immunogenetics 2000;51:659–669. [PubMed: 10941837]
18. Luke MM, O'Meara ES, Rowland CM, Shiffman D, Bare LA, Arellano AR, Longstreth WT Jr, Lumley T, Rice K, Tracy RP, Devlin JJ, Psaty BM. Gene variants associated with ischemic stroke: the cardiovascular health study. Stroke 2009;40:363–368. [PubMed: 19023099]
19. Maltais LJ, Lovering RC, Taranin AV, Colonna M, Ravetch JV, Dalla-Favera R, Burrows PD, Cooper MD, Davis RS. New nomenclature for Fc receptor-like molecules. Nat.Immunol 2006;7:431–432. [PubMed: 16622424]
20. Maruoka T, Nagata T, Kasahara M. Identification of the rat IgA Fc receptor encoded in the leukocyte receptor complex. Immunogenetics 2004;55:712–716. [PubMed: 14647992]
21. Masuda K, Davis RS, Maruyama T, Zhang J, He T, Cooper MD, Wang J, Burrows PD. FcRY, an Fc receptor related gene differentially expressed during B lymphocyte development and activation. Gene 2005;363:32–40. [PubMed: 16263223]
22. Masuda K, Ouchida R, Hikida M, Nakayama M, Ohara O, Kurosaki T, Wang J. Absence of DNA polymerase theta results in decreased somatic hypermutation frequency and altered mutation patterns in Ig genes. DNA Repair (Amst) 2006;5:1384–1391. [PubMed: 16890500]
23. Masuda K, Ouchida R, Li Y, Gao X, Mori H, Wang JY. A critical role for REV1 in regulating the induction of C:G transitions and A:T mutations during Ig gene hypermutation. J.Immunol 2009;183:1846–1850. [PubMed: 19587019]

24. Masuda K, Ouchida R, Yokoi M, Hanaoka F, Azuma T, Wang JY. DNA polymerase eta is a limiting factor for A:T mutations in Ig genes and contributes to antibody affinity maturation. Eur.J.Immunol 2008;38:2796–2805. [PubMed: 18825746]
25. Mechetina LV, Najakshin AM, Volkova OY, Guselnikov SV, Faizulin RZ, Alabyev BY, Chikaev NA, Vinogradova MS, Taranin AV. FCRL, a novel member of the leukocyte Fc receptor family possesses unique structural features. Eur.J.Immunol 2002;32:87–96. [PubMed: 11754007]
26. Monteiro RC, Van De Winkel JG. IgA Fc receptors. Annu.Rev.Immunol 2003;21:177–204. [PubMed: 12524384]
27. Niederer HA, Clatworthy MR, Willcocks LC, Smith KG. FcgammaRIIB, FcgammaRIIIB, and systemic lupus erythematosus. Ann.N.Y.Acad.Sci 2010;1183:69–88. [PubMed: 20146709]
28. Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. Nat.Rev.Immunol 2008;8:34–47. [PubMed: 18064051]
29. Norderhaug IN, Johansen FE, Schjerven H, Brandtzaeg P. Regulation of the formation and external transport of secretory immunoglobulins. Crit Rev.Immunol 1999;19:481–508. [PubMed: 10647747]
30. Pritchard NR, Cutler AJ, Uribe S, Chadban SJ, Morley BJ, Smith KG. Autoimmune-prone mice share a promoter haplotype associated with reduced expression and function of the Fc receptor FcgammaRII. Curr.Biol 2000;10:227–230. [PubMed: 10704418]
31. Rahman ZS, Manser T. Failed up-regulation of the inhibitory IgG Fc receptor Fc gamma RIIB on germinal center B cells in autoimmune-prone mice is not associated with deletion polymorphisms in the promoter region of the Fc gamma RIIB gene. J.Immunol 2005;175:1440–1449. [PubMed: 16034080]
32. Ravetch JV, Bolland S. IgG Fc receptors. Annu.Rev.Immunol 2001;19:275–290. [PubMed: 11244038]
33. Schreeder DM, Cannon JP, Wu J, Li R, Shakhmatov MA, Davis RS. Cutting Edge: FcR-Like 6 Is an MHC Class II Receptor. J.Immunol. 2010
34. Shibuya A, Sakamoto N, Shimizu Y, Shibuya K, Osawa M, Hiroyama T, Eyre HJ, Sutherland GR, Endo Y, Fujita T, Miyabayashi T, Sakano S, Tsuji T, Nakayama E, Phillips JH, Lanier LL, Nakauchi H. Fcα/μ receptor mediates endocytosis of IgM-coated microbes. Nat.Immunol 2000;1:441–446. [PubMed: 11062505]
35. Shiffman D, O'Meara ES, Bare LA, Rowland CM, Louie JZ, Arellano AR, Lumley T, Rice K, Iakoubova O, Luke MM, Young BA, Malloy MJ, Kane JP, Ellis SG, Tracy RP, Devlin JJ, Psaty BM. Association of gene variants with incident myocardial infarction in the Cardiovascular Health Study. Arterioscler.Thromb.Vasc.Biol 2008;28:173–179. [PubMed: 17975119]
36. Shima H, Takatsu H, Fukuda S, Ohmae M, Hase K, Kubagawa H, Wang JY, Ohno H. Identification of TOSO/FAIM3 as an Fc receptor for IgM. Int.Immunol 2010;22:149–156. [PubMed: 20042454]
37. Su K, Wu J, Edberg JC, Li X, Ferguson P, Cooper GS, Langefeld CD, Kimberly RP. A promoter haplotype of the immunoreceptor tyrosine-based inhibitory motif-bearing FcgammaRIIb alters receptor expression and associates with autoimmunity. I. Regulatory FCGR2B polymorphisms and their association with systemic lupus erythematosus. J.Immunol 2004;172:7186–7191. [PubMed: 15153543]
38. Trowsdale J, Barten R, Haude A, Stewart CA, Beck S, Wilson MJ. The genomic context of natural killer receptor extended gene families. Immunol.Rev 2001;181:20–38. [PubMed: 11513141]
39. Wende H, Colonna M, Ziegler A, Volz A. Organization of the leukocyte receptor cluster (LRC) on human chromosome 19q13.4. Mamm.Genome 1999;10:154–160. [PubMed: 9922396]
40. Wilson TJ, Colonna M. A new Fc receptor homolog, FREB2, found in germinal center B cells. Genes Immun 2005;6:341–346. [PubMed: 15815692]
41. Xiu Y, Nakamura K, Abe M, Li N, Wen XS, Jiang Y, Zhang D, Tsurui H, Matsuoka S, Hamano Y, Fujii H, Ono M, Takai T, Shimokawa T, Ra C, Shirai T, Hirose S. Transcriptional regulation of Fcgr2b gene by polymorphic promoter region and its contribution to humoral immune responses. J.Immunol 2002;169:4340–4346. [PubMed: 12370366]

Fig. 1A

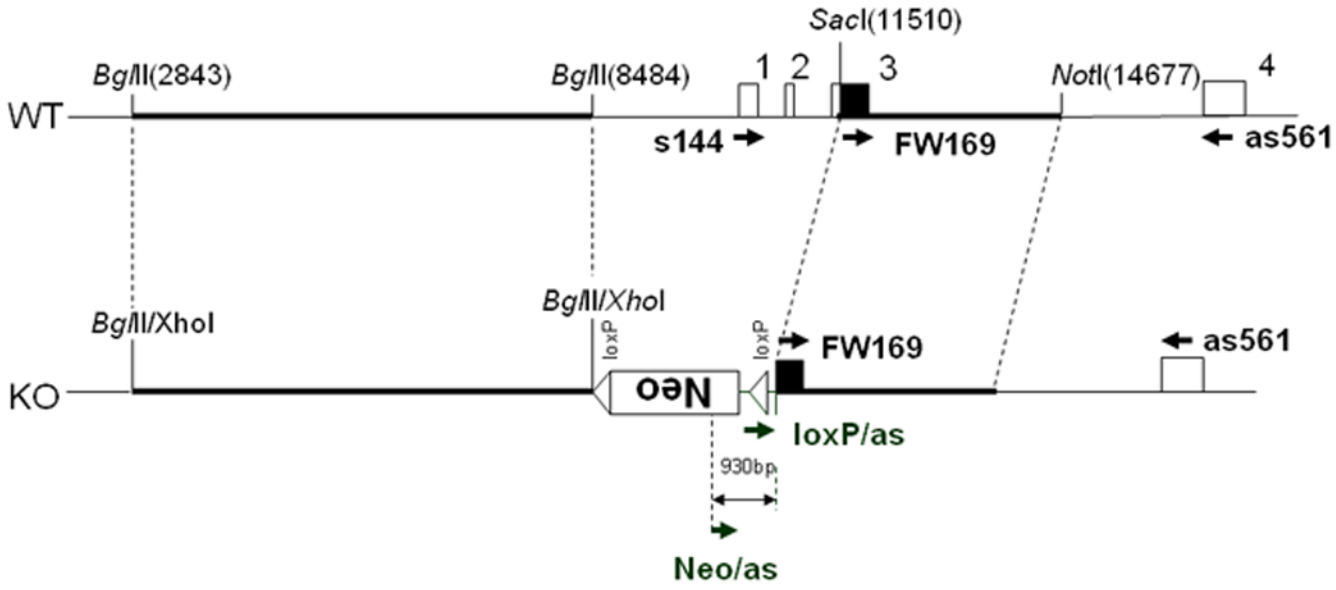

Fig. 1B

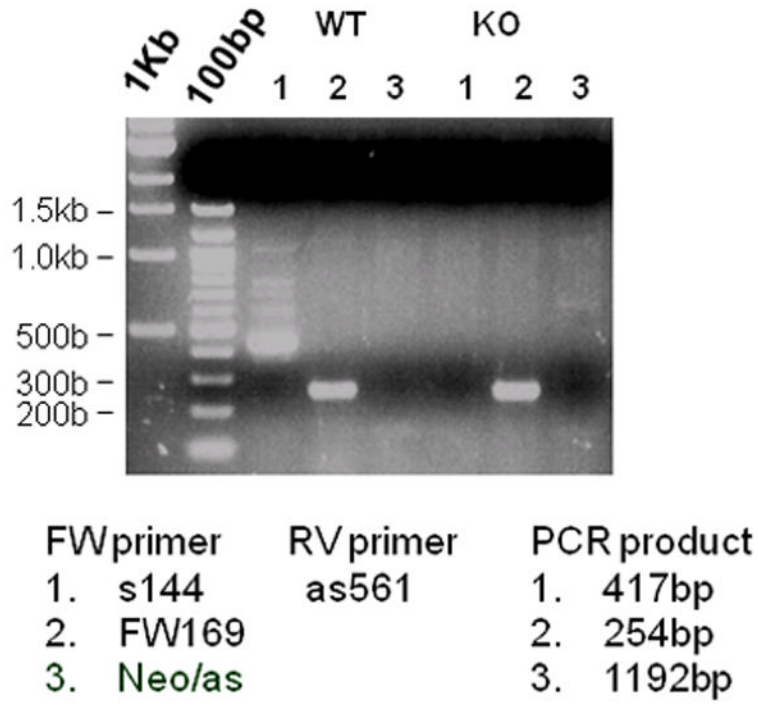

Fig. 1. *Fcrlb* targeting strategy and expression of *Fcrlb* mRNA

(A) Schematic representation of the wild type (WT) and targeted (KO) *Fcrlb* alleles. A detailed description of the targeting vector can be found in Fig. S1. Primers used for RT-PCR analysis of *Fcrlb* mRNA expression are illustrated by arrowheads. **(B)** RT-PCR analysis of *Fcrlb* gene expression in splenic B cells. Three different forward (FW) primers in conjunction with a common reverse (RV) primer were used in RT-PCR analysis of *Fcrlb* expression in purified splenic B cells from WT and KO mice. The expected sizes of the PCR products are indicated.

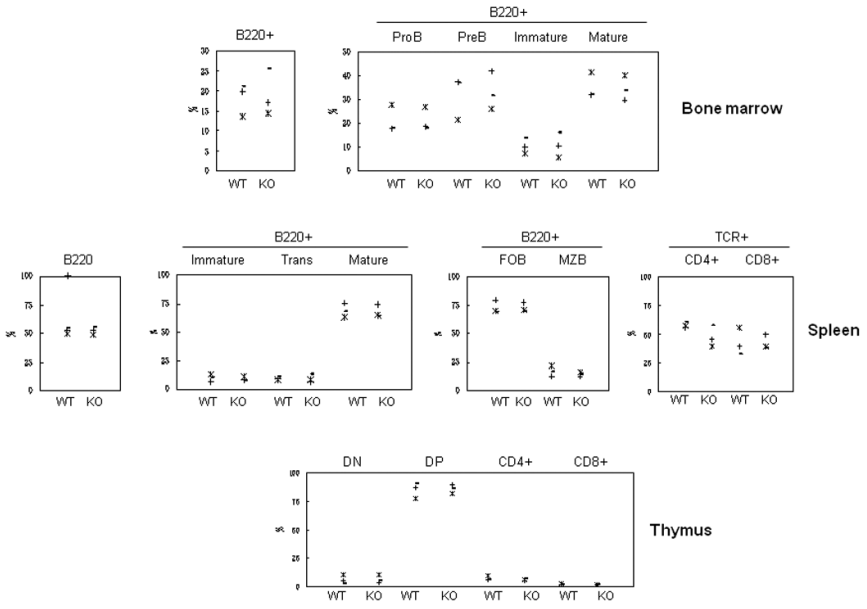

Fig. 2. Analysis of lymphocyte subpopulations by flow cytometry

The frequency of the various lymphoid subpopulations was analyzed by flow cytometry using the markers described in the Materials and Methods. B cell populations analyzed were: Bone marrow, total $B220^+$, proB, preB, immature and mature B; Spleen, total $B220^+$, immature, transitional (Trans) and mature B, follicular B (FOB) and marginal zone B (MZB). T cell populations analyzed were: Spleen, $CD4^+$ and $CD8^+$; thymus, CD4 and CD8 double negative (DN) and double positive (DP), $CD4^+$ and $CD8^+$ single positive. Each symbol represents one mouse. Three pairs of mice were analyzed in these experiments. Representative flow cytometry profiles are shown in Fig. S3.

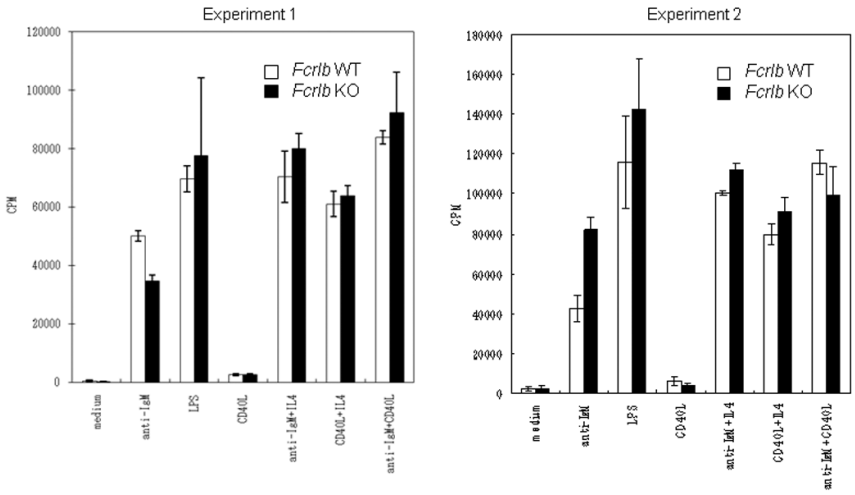

Fig. 3. *In vitro* B cell proliferation

Purified splenic B cells were stimulated with anti-IgM, LPS, and CD40L, as well as with the indicated combinations of these stimuli. The proliferative response of KO and WT B cells was analyzed at 48 hours. Data from two independent experiments, each done with a pair of WT and KO mice are depicted. The bars indicate the standard deviation of four assay wells.

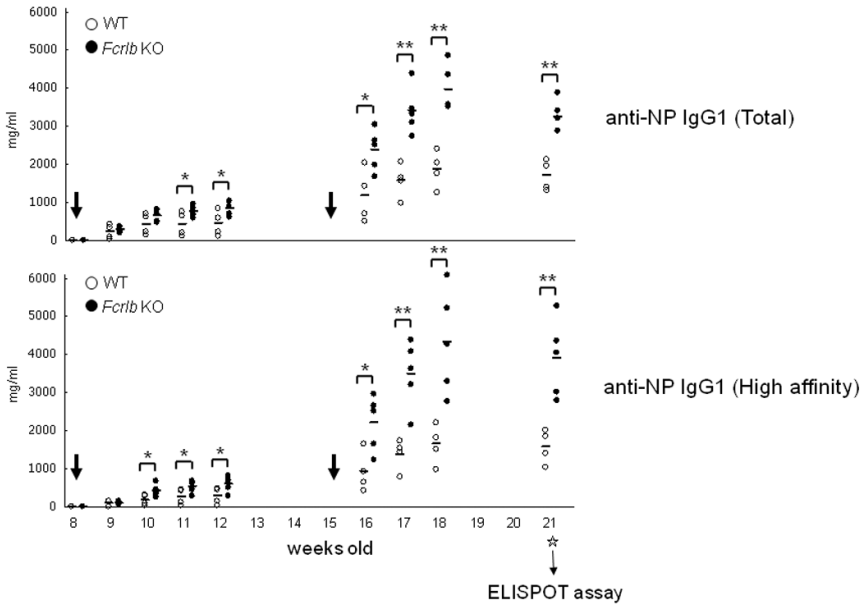

Fig. 4. Primary and secondary antibody responses after immunization with $NP_{16}CGG$

Mice were immunized when they were 8 weeks old and boosted at 15 weeks (indicated by the large arrows) with alum precipitated $NP_{16}CGG$. Serum samples were collected at the indicated time points for analysis of total and high affinity NP-antibodies by an ELISA assay. Six weeks after the second immunization the mice were sacrificed and antibody secreting cells in spleen and bone marrow were enumerated by an ELISPOT assay (Fig. 5). Each symbol represents one mouse. *, $p < 0.05$; **, $p < 0.01$ (unpaired t-test).

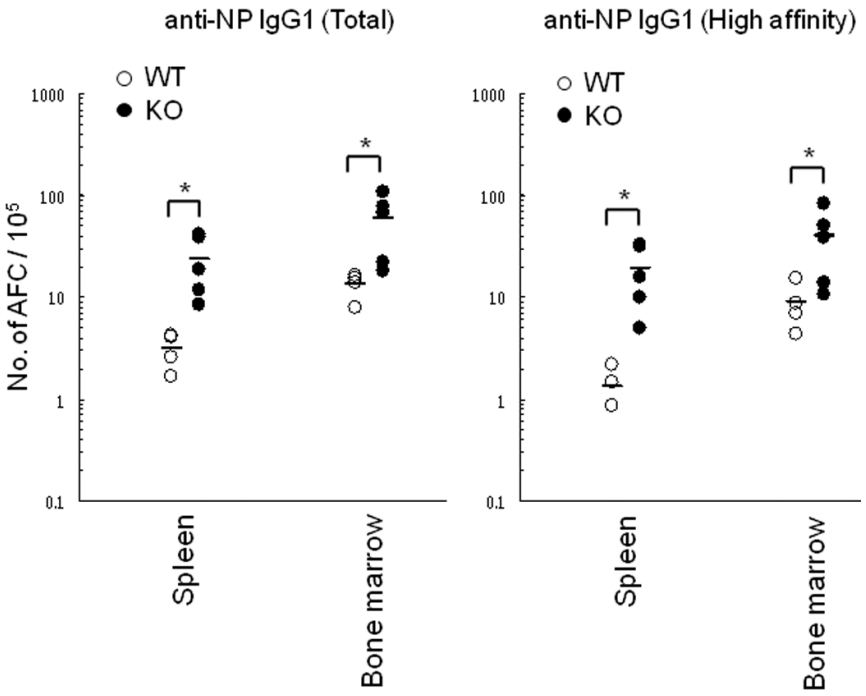

Fig. 5. Enumeration of NP-specific antibody forming cells by ELISA in WT and KO mice

The frequency of antibody-forming cells (AFC) was determined six weeks after the secondary immunization with $NP_{16}CGG$. Both total and high affinity IgG1 anti-NP AFC were increased in the *Fcrlb* KO mice. Each symbol represents one mouse. *, $p < 0.05$ (unpaired t-test).

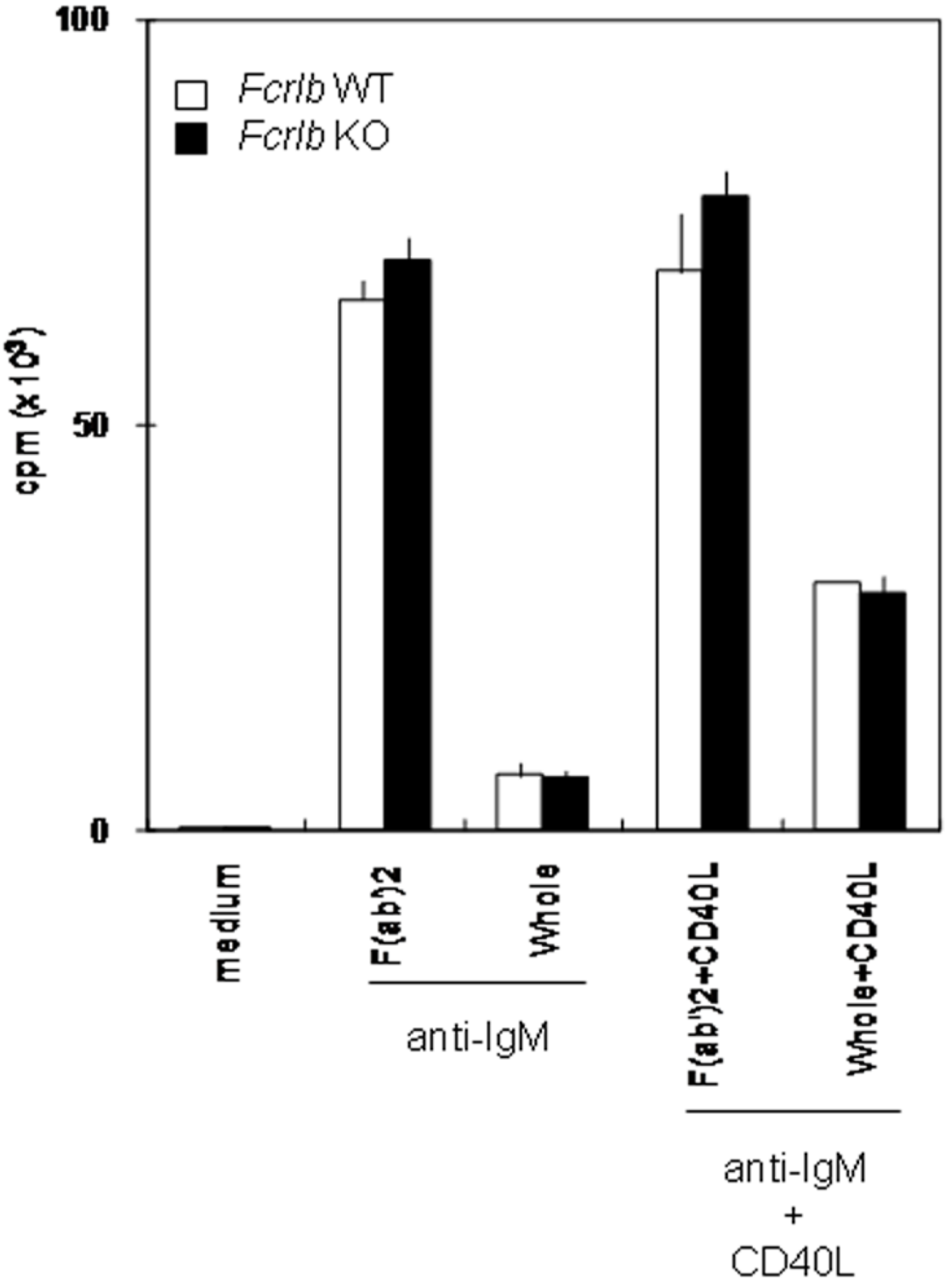

Fig. 6. FcγRIIb-mediated inhibition of BCR induced B cell proliferation is intact in *Fcrlb* KO mice

Purified splenic B cells from WT or *Fcrlb* KO mice were stimulated with F(ab'$_2$) anti-IgM antibodies or with intact anti-IgM antibodies (to co-engage the BCR and FcγRIIb) in the presence or absence of CD40 ligand (CD40L). Proliferation was measured at 48 hours.

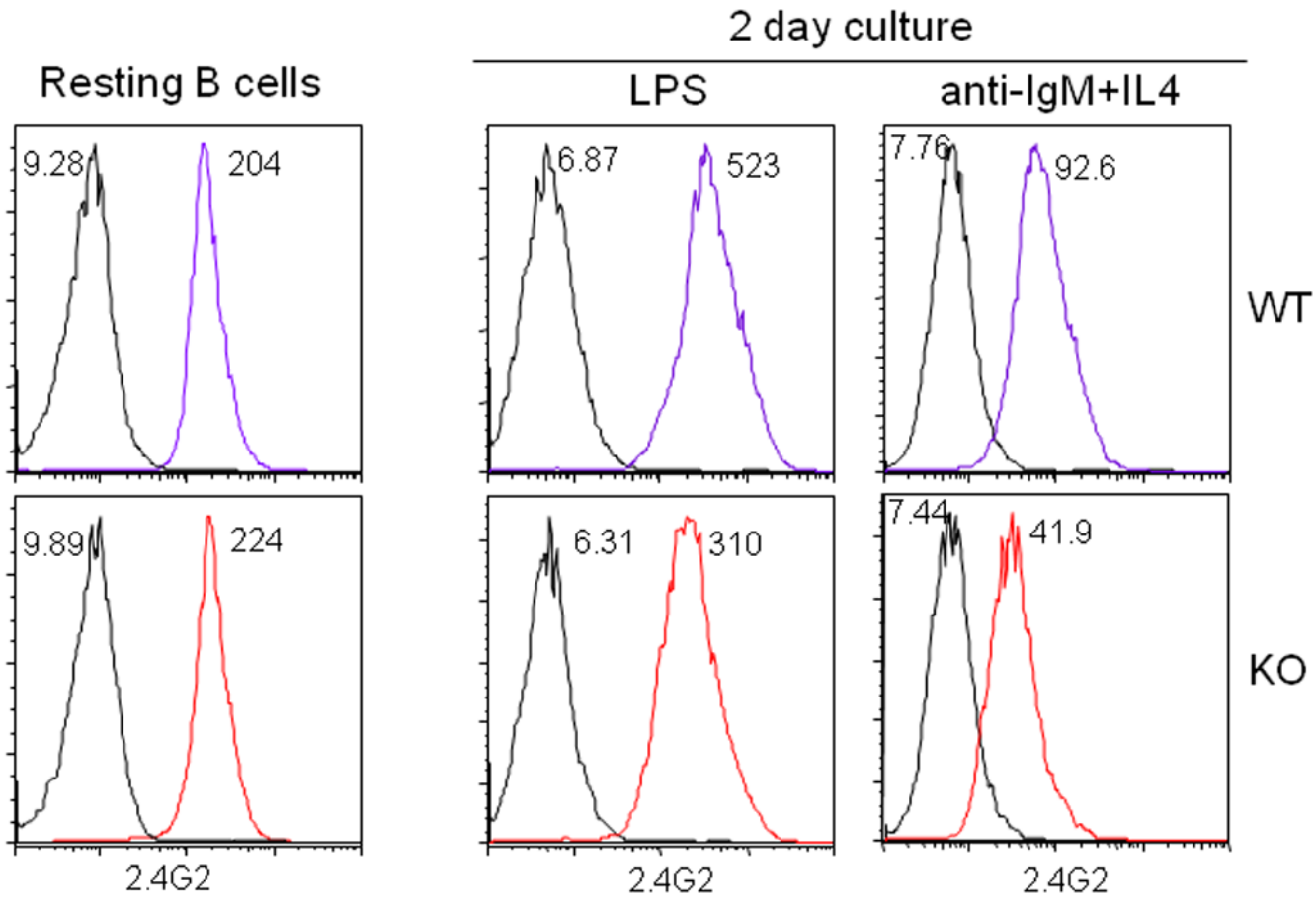

Fig. 7. FcγRIIb expression by splenic B cells before and after stimulation

Purified splenic B cells were analyzed by flow cytometry for FcγRIIb expression using the 2.4G2 mAb. FcγRIIb expression on resting B cells was identical in WT and KO mice, however it was lower in the KO B cells after stimulation with LPS or anti-IgM plus IL4.

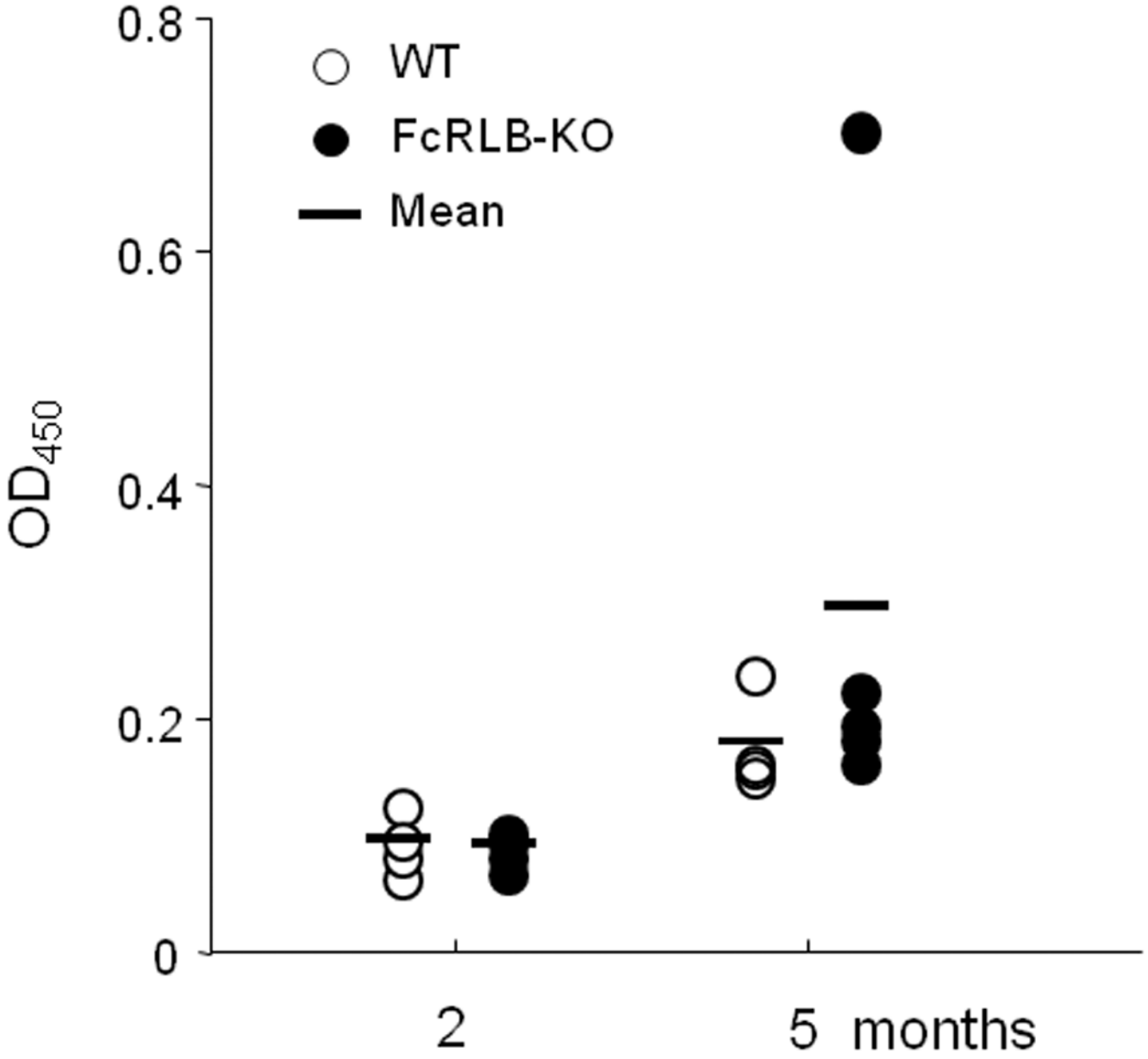

Fig. 8. Anti-nuclear antibody titers

Serum samples from wildtype (open circles) and *Fcrlb*−/− (filled circles) mice at two and five months of age were analyzed for ANA titers by ELISA. Data from individual mice are depicted.