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Differential Expression of the Inhibitory IgG Fc Receptor FcyRIIB on Germinal Center Cells: Implications for Selection of High-Affinity B Cells

Sambasiva P. Rao, Kalpit A. Vora, and Tim Manser

Receptors for the Fc portion of IgG (FcyR) are widely distributed on hematopoietic cells and play an important role in immune regulation by linking Ab-mediated responses with effector functions. The murine low-affinity receptor for IgG, FcyRIIB, mediates inhibition of B cell receptor-triggered events in primary B cells. We investigated the expression of FcyRIIB on germinal center (GC) cells to better understand its role in memory B cell development. Immunohistological analyses demonstrated differential regulation of FcyRIIB on GC cells. Its levels are markedly down-regulated on GC B cells and up-regulated on follicular dendritic cells (FDC) at all times during the GC response. Analyses of surface expression of FcyRIIB by flow cytometry and FcyRIIB mRNA levels by RT-PCR analysis confirmed that this FcR is down-regulated in GC B cells. In mice lacking FcyRIIB, the development of the secondary FDC reticulum in GCs is substantially delayed, although the overall kinetics of the GC response are unaltered. These findings have direct implications for models proposed to account for the selection of high-affinity B cells in the GC and suggest a role for FcyRIIB in promoting the maturation of the FDC reticulum.

mRNA and protein expression are significantly down-regulated in GC B cells as compared with non-GC B cells. In addition, whereas the kinetics and magnitude of the GC response appear unaltered in FcγRIIB-deficient mice, the development of FDC reticula in GCs is delayed, supporting a role for this receptor in FDC maturation. The implications of these results are discussed in the context of models proposed to account for selection of high-affinity B cells in the GC.

**Materials and Methods**

**FcγRIIB−/− mice** on a C57BL/6 background (20) and C57BL/6 mice were purchased from Taconic Farms (Germantown, NY). The generation and characterization of common γ-chain-deficient mice have been described previously (21). These mice, obtained from Dr. J. Ravetch (Rockefeller University, New York, NY), had been backcrossed to C57BL/6 mice for eight generations. All mice were maintained in pathogen-free conditions. Mice (10–12 wk old) were immunized i.p. with 3 × 10⁷ SRBC (Lampire Biological Labs, Pipersville, PA) in PBS or 100 µg 4-hydroxy-3-nitrophe- nyl acetyl-chicken γ-globulin (NP 13-CGG) in alum and were sacrificed at different time intervals for analysis.

**Antibodies**

The following Abs were used for immunohistochemistry or flow cytometric analysis: PE-Texas Red-conjugated anti-B220 (clone RA3-6B2; Caltag Laboratories, Burlingame, CA); FITC-labeled GL7; PE-labeled anti-FcγRIIFcyRIII (clone 2.4G2); PE-labeled anti-B220 (clone RA3-6B2); biotin-labeled anti-CD35 (clone SC12) (all purchased from BD PharMingen, San Diego, CA); unconjugated rat IgG Ab to mouse FDCs (FDC-M1 and FDC-M2; gifts from Dr. M. Kosco-Vilbois, Serono Pharmaceutical Research Institute, Plan-les-Quates, Switzerland); FITC-labeled MOMA-2 (S erotec, Oxford, U.K.); biotin-labeled (Fab'); mouse anti-rat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA); biotin-labeled peanut agglutinin (PNA; Sigma-Aldrich, St. Louis, MO) or PE or biotin-labeled anti-IgD (clone 11-26; Southern Biotechnology Associates, Birmingham, AL) and streptavidin (SA)-PE (Molecular Probes, Eugene, OR). The K9.361 hybridoma was a kind gift from Dr. U. Hammerling (Sloan Kettering Memorial Hospital, New York, NY).

**Histology**

Spleens from immunized mice were obtained at various times after immunization and processed for histological analysis as previously described (22). Briefly, spleens were embedded in Tissue-Tek OCT compound (Fisher Scientific, Bridgeport, NJ) by freezing in a 2-methylbutane bath cooled with liquid nitrogen. Frozen spleens were stored at −70°C until sectioned. Sections of 6 µm were cut on a cryostat microtome and mounted onto 0.01% poly-L-lysine (Sigma-Aldrich)-coated slides. The sections were air dried for 1 h, fixed in acetone for 15 min, air dried again for at least 2 h, and stored at −70°C until further analysis.

Frozen sections were rehydrated in TBS for 20 min followed by blocking in TBS, 5% BSA, 0.05% Tween 20 for another 20 min. Double immunohistology was then performed by staining with fluorescent Abs described above for 30 min. For unconjugated Abs, as a secondary step, sections were stained for 30 min with biotinylated mouse anti-rat Ab. The slides were then washed three times in TBS, 5% BSA, 0.05% Tween 20, and the biotinylated Abs were revealed by SA-PE. The slides were washed and stored in PBS in the dark. The stained sections were analyzed using a fluorescence microscope (Leitz Diaplan, Leitz, Wetzlar, West Germany), and digital images were captured using a Kodak DC290 Zoom digital camera and MDS-290 software (Eastman Kodak, Rochester, NY).

**Flow cytometry and cell sorting**

Cell suspensions prepared from spleens excised from mice on day 8 post-SRBC immunization were depleted of erythrocytes with ammonium chloride Tris lysis (23). For four-color surface staining, 2 × 10⁶ cells suspended in PBS containing 2% BSA were incubated with pretitrated dilutions of GL7-FITC, biotinylated anti-IgD, anti-B220-PE-Texas Red, and 2.4G2-PE for 30 min at 4°C. A SA Red 670 was used as a second-step reagent. Cells were analyzed using a Coulter Epics XL/MCL analyzer, and the GL7-FITC was amplified using primers purchased from Clontech Laboratories (Palo Alto, CA). RT-PCR was conducted in 50-µl volumes with one cycle programmed to perform cDNA synthesis at 50°C for 30 min and 94°C for 2 min, followed immediately by PCR amplification for 30 cycles at 95°C for 1 min, 55°C for 45 s, and 72°C for 1 min. A final extension was done at 72°C for 5 min. One-fifth volume of the PCR was electrophoresed on a 1.5% agarose gel, and the bands were visualized under UV light.

**Gel hybridization**

Agarose gels were dried at 65°C for 2 h under vacuum, denatured in 0.5 M NaOH, 1.5 M NaCl for 1 h, and neutralized for 1 h in 0.5 M Tris, 3 M NaCl. The gels were then prehybridized at 42°C in prehybridization buffer solution containing 6× 0.6 M NaCl, 0.15 M Tris (pH 8.0), 6 mM EDTA; 0.05 M Denhardt’s solution; 0.1% SDS; and 250 µg of herring sperm DNA. Tapes were prepared from PCR products by 7° labeling using a random primed DNA labeling kit (Roche Molecular Biochemicals, Mannheim, Germany), denatured, and added to the prehybridization buffer, and the gels were hybridized overnight at 42°C. The gels were washed at low stringency in 5× SSC, 5% SDS for 1 h followed by two 30-min washes at 43°C. Subsequently, a high stringency wash in 0.1× SSC, 0.1% SDS at 60°C was performed. The gels were scanned on a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) after overnight exposure. The bands were quantitated after subtracting the background signal using Image Quant software (Molecular Dynamics). The integrated intensity of each band representing individual PCR products was measured and normalized using the β-actin band intensity for each individual sample.

**Results**

**Kinetics of the GC response to SRBC immunization**

SRBC have been previously shown to induce robust GC responses in an adjuvant-independent manner (24, 25). However, only limited information was available regarding the magnitude and kinetics of this response. Therefore, we first examined the number and sizes of GC in the spleens of SRBC immunized C57BL/6 mice. Spleen sections obtained from days 4–40 after immunization were stained with GL7. The total number of GCs in each section were counted and categorized into three different sizes by counting GL7⁺ cells as previously described (26). A summary of the results is presented in Fig. 1. On day 4 after SRBC immunization, the majority of GL7⁺ cells were scattered in follicles. Small foci of GL7⁺ cells localized within the FDC network as well as a few well-developed small GCs were detectable (data not shown). By day 6, there was a large expansion in the number of GC comprising all sizes. This large number of GCs was maintained until day 12, after which the numbers declined. Large GCs were present from day 6, reached a peak by day 10, and thereafter declined with a corresponding increase in medium and small GCs. Although the number of medium sized GCs started to decline after day 12, the small GCs continued to increase, perhaps due to the dissolution of some of the large and medium sized GCs. The numbers of small GC gradually declined from day 16 onward, and by day 40 only a few GCs were present, almost all of which were small.

**GL7 and PNA staining of GC B cells**

Although a strong correlation exists between the patterns of PNA and GL7 staining, earlier studies reported the presence of both PNA⁺GL7⁺ as well as PNA⁺GL7⁻ cell populations in spleen (27, 28). Immunohistological analysis of spleen sections from C57BL/6
mice derived 8 days after primary SRBC immunization was performed by staining with GL7 and PNA. As shown in the inset in Fig. 2, most PNA$^+$ cells are GL7$^+$ (and thus appear yellow), although some areas stained positive for either GL7 only or PNA only, suggesting heterogeneity in the expression of their respective ligands. Further, a rim of PNA$^+$ cells that were GL7$^-$ (Fig. 2, arrow) was also detected surrounding the GC. Although the significance of these differences in the staining pattern of GL7 and PNA is presently unclear, the results show that there is a more restricted pattern of expression of the ligands for GL7 than of the ligands for PNA in GCs. Therefore, we used GL7 as a marker to define GC cells in our subsequent analyses.

Differential regulation of FcγRIIB expression on GC cells

We next examined whether there was any difference in the level of FcγRIIB expression on GC and follicular B cells. In contrast to macrophage/monocyte lineage cells, B cells exclusively express the b1 isoform of FcγRIIB and have been reported to lack expression of other Fcγ family receptors such as FcγRI, FcγRIII, and the b2 isoform of FcγRII (1, 3). The mAb 2.4G2 recognizes the extracellular domains of FcγRII and FcγRIII. To avoid FcγRIII staining with 2.4G2, we immunized Fcγ chain$^-$ mice with SRBC. These mice express neither FcγRI nor FcγRIII due to the absence of the common γ-chain, required for assembly and membrane transport of these receptors. However, FcγRIIB expression is not perturbed in these mice. Immunohistological analysis of spleen sections from Fcγ chain$^-$ mice that were immunized with SRBC 8 days earlier was performed using 2.4G2 in combination with GL7 and anti-B220. Representative images are shown in Fig. 2. As shown in Fig. 2B, follicular mantle B cells clearly stain with 2.4G2, and the marginal zone stains even more strongly with 2.4G2, suggesting relatively high levels of FcγRIIB expression.
GL7+ GCs revealed an interesting pattern of 2.4G2 staining. Although FcγRIIB expression was undetectable on B cells toward one side of the GC, intense 2.4G2 staining was observed polarized to the other side of the GC, suggesting up-regulated FcγRIIB expression in this area. Similar results were obtained when sections were stained with PNA and 2.4G2 (Fig. 2A). This sectored pattern of FcγRIIB expression was consistently observed. Parallel sections stained with Ab to B220 and 2.4G2 were then analyzed. As shown in Fig. 2C, B220+ marginal zone cells exhibited relatively high levels of FcγRIIB (and thus appear orange in Fig. 2C), and the B220+ follicular mantle cells also coexpressed FcγRIIB (and thus appear yellow in Fig. 2C). In contrast, the B220+ areas corresponding to GC exhibited sectored, with undetectable levels of FcγRIIB in one area (thus appearing green; Fig. 2C, arrow) and high levels of FcγRIIB in the other area.

We then examined whether the observed differential regulation of FcγRIIB was also a feature of GC responses to the well-characterized model Ag, NP. Day 10 spleen sections from NP,CGG-immunized Fcγ chain−/− mice were stained with 2.4G2 and GL7 or anti-B220. These experiments also showed sectored, with high levels of FcγRIIB in one region and low to undetectable levels in the other region of GCs (Fig. 2D and E). The analysis was further extended by using an FcγRIIB-specific mAb, K9.361 (29–31), that was made available to us at a later time during the study. K9.361 exclusively recognizes FcγRIIB (Ly17.2 allotype of Ly17.1/Ly17.2 alloantigen system) in C57BL/6 mice and does not bind to FcγRIII (31). Immunohistological analysis of day 8 spleen sections from SRBC-immunized C57BL/6 mice with K9.361 and GL7 or anti-B220 revealed exactly the same sectored pattern of GC staining observed with 2.4G2 (Fig. 2, F and G). These data collectively indicate that the expression of FcγRIIB is differentially modulated in these two areas of GCs.

FcγRIIB expression is markedly reduced on GC B cells and up-regulated on FDC

We previously demonstrated that FcγRIIB is up-regulated on FDC within the GC (16). In the experiments described above (Fig. 2), we could not evaluate whether the GC B cells present in the FDC-rich regions of the GC also expressed FcγRIIB. To investigate this issue, chimeric mice were created by lethally irradiating FcγRIIB−/− mice and reconstituting them with bone marrow from FcγRIIB+/+ C57BL/6 mice. Because FDCs are resistant to radiation (32), they are derived from the host in these chimeras and therefore cannot express FcγRIIB. The donor cells, including B cells, however, can express the FcγRIIB receptor. Day 9 spleen sections from NP-CGG-immunized chimeric mice were examined for expression of FcγRIIB in GCs. In the absence of FcγRIIB expression on FDC, any staining of GC cells with the 2.4G2 Ab should result from FcR expression on B cells. As shown in Fig. 3, although FDCs were present in the GC, there was a lower level of FDC-M1 staining in general (Fig. 3A) and as expected, 2.4G2 staining on FDC was absent. B cells in these GCs, including those present in the FDC reticulum; exhibited markedly reduced levels of 2.4G2 staining compared with mantle zone B cells (Fig. 3B).

GC B cells express low to undetectable levels of FcγRIIB at all stages of the GC response

We further investigated whether reduced expression of FcγRIIB on GC B cells is characteristic of all stages of the GC response. FcγRIIB expression was evaluated on SRBC-immunized C57BL/6 mice from day 4 to day 30 and day 4 to day 16 in Fcγ chain−/− mice. Spleen sections were stained with GL7 in combination with 2.4G2, FDC-M1, or FDC-M2. Representative images are shown in Fig. 4. The results of this analysis revealed “sectoring” of GC due to up-regulated FcγRIIB levels in the region rich in FDCs and low to undetectable levels of FcγRIIB expression on GC B cells where the FDC network was absent. These observations were made at all time points of the GC response starting from day 4 when GC clusters first appeared (Fig. 4A). Furthermore, this was consistent for all GC sizes.

In Fcγ chain−/− mice, we observed an accelerated kinetics of the GC response. In contrast to C57BL/6 mice, there were many large GCs by day 4, and by day 16 most of the GCs were small and the numbers were substantially reduced (data not shown). These changes in GC kinetics might be due to reduced clearance and increased deposition of immune complexes in Fcγ chain−/− mice as we previously reported (22). FDC organization and development, however, appeared normal in these mice (Fig. 4B). Consistent with the findings in C57BL/6 mice (Fig. 4A), the GCs in Fcγ chain−/− mice exhibited sectoring, with increased levels of FcγRIIB on FDC and low to undetectable levels on GC B cells in FDC-deficient areas at all time points (Fig. 4B).

Flow cytometric and RT-PCR analysis of FcγRIIB expression in GC B cells

As an alternative assay for FcγRIIB expression on splenic GC B cells, we used flow cytometry. The mAbs 2.4G2 and K9.361 were used to determine the levels of FcγRIIB on B cells from Fcγ chain−/− and C57BL/6 mice, respectively. Four-color immunofluorescence analysis was performed, and surface expression of FcγRIIB was analyzed on B220+ gated GL7−IgD− (GC) and GL7+IgD− (non-GC) B cells. As shown in Fig. 5, compared with the GL7+IgD+ non-GC B cell population, there was a substantial decrease of FcγRIIB expression on GL7−IgD− GC B cells. The

**FIGURE 3.** FcγRIIB is down-regulated on all GC B cells including those in FDC-rich areas. Chimeric mice created by lethally irradiating FcγRIIB−/− mice and reconstituting them with bone marrow from FcγRIIB+/+ C57BL/6 mice were immunized with NP-CGG. Sections prepared from spleens derived on day 9 postimmunization were examined for expression of FcγRIIB in GCs. Two-color staining was performed on parallel sections with GL7-FITC and FDC-M1, or GL7-PTC and 2.4G2-PE. FDC-M1 was revealed with secondary biotin-labeled mouse F(ab')2 anti-rat IgG followed by SA-PE. Shown are representative images of GCs in parallel sections. The FDC reticulum (A) is not as well developed as in normal mice, and 2.4G2 staining on B cells in FDC-rich regions is very low to undetectable (B). Original magnification, × 200.
FIGURE 4. FcγRIIB expression is low to undetectable on GC B cells in FDC poor regions throughout the GC response. Day 4 to day 30 parallel spleen sections derived from C57BL/6 (A) and day 4 to day 16 parallel spleen sections from Fcγ chain−/− mice (B) after SRBC immunization were stained with GL7-FITC in combination with 2.4G2-PE, FDC-M1, or FDC-M2. FDC-M1 and FDC-M2 were revealed with secondary biotin-labeled mouse F(ab′)2 anti-rat IgG followed by SA-PE. Note that the pattern of FcγRIIB and FDC-M1 staining results in sectoring the GC into two discrete regions at all time points of the response. Original magnification, × 200.
extent of surface FcγRIIB down-regulation on GC B cells detected with 2.4G2 (Fig. 5A) and K9.361 (Fig. 5B) was similar, thus confirming our immunohistological observations (Fig. 2). It is possible that due to the differences in the sensitivities of flow cytometry and fluorescence microscopy techniques, low level expression of FcγRIIB on GC B cells was not detectable in immunohistological analyses. However, the unimodal distribution of FcγRIIB staining indicates that all GC B cells exhibit this low expression level with no evidence for the presence of any minor population of GC B cells that express FcγRIIB at levels similar to GL7− IgD+ B cells.

To examine the mechanism and the extent of down-regulation of FcγRIIB, RNA prepared from sorted GL7−IgD− GC cells from immunized C57BL/6 mice was analyzed using RT-PCR to determine the levels of FcγRIIB encoding mRNA. These levels were compared with those in the GL7− IgD−-naïve B cell population. FcγRIIB mRNA from both cell populations was amplified using gene-specific primers (see Materials and Methods). β-Actin mRNA served as an internal control to ensure that equal quantities of cDNA were amplified in the reactions. To test the linearity of PCR amplification and the extent of down-regulation, PCR products removed after different numbers of PCR cycles were subjected to agarose gel electrophoresis. The gel was hybridized with an FcγRIIB-specific probe and analyzed by PhosphorImager and expressed as units of integrated band intensity (see Materials and Methods). The results of this analysis showed nearly a 6-fold reduction of FcγRIIB mRNA in GC B cells compared with non-GC IgD+ B cells (Fig. 6).

**FDC exhibit maturation defects in FcγRIIB-deficient mice**

The differential regulation of FcγRIIB on GC B cells and FDC prompted us to examine the GC response in mice lacking this receptor. Histological analysis of spleen sections from day 6 to day 22 post-SRBC immunization was performed on FcγRIIB−/− and C57BL/6 mice with GL7, FDC-M1, and FDC-M2. In C57BL/6 mice, the GC FDC reticulum was seen as fine processes extending throughout one side of the GC (see Fig. 4). This was true at all time points after immunization. In contrast, as shown in Fig. 7, this reticular pattern of staining was very weak in FcγRIIB-deficient GCs at early and intermediate times in the GC response. In these GCs, strong FDC-M1 and FDC-M2 staining exhibited a largely punctate pattern until day 16. Thereafter, FDC staining was similar to that seen in C57BL/6 mice. The primary FDC reticula in FcγRIIB−/− mice, however, appeared normal at all stages of the GC response as judged by staining with the anti-CD35 Ab-8C12. Interestingly, even the punctate pattern of FDC staining observed during the early GC response of FcγRIIB−/− mice was largely restricted to one side of the GC. To investigate the cellular origin of this punctate staining, parallel sections from the experiment illustrated in Fig. 7 were stained with GL7, FDC-M1, and the MOMA-2 mAb. MOMA-2 has been previously reported to detect monocytes and tingible body macrophages (33). Representative images from this analysis are shown in Fig. 8. At early time points in FcγRIIB−/− mice (day 8 is illustrated in Fig. 8), MOMA-2 and FDC-M1 staining were largely colocalized to the punctate bodies, and these were rare in C57BL/6 GCs. At later times (day 20 is illustrated), whereas the reticular pattern of FDC-M1 staining in FcγRIIB−/− GCs appeared similar to C57BL/6 GCs, MOMA-2/FDC-M1 double-positive cells were still clearly visible but remained rare in C57BL/6 GCs. Interestingly, we also observed MOMA-2 positive, FDC-M1 negative cells in the GCs of FcγRIIB−/− mice at these later time points.

To test whether the altered pattern of FDC-M1 intra-GC staining characteristic of the early stages of the GC response in FcγRIIB−/− mice correlated with a difference in the time course of the GC response, the kinetics of this response were examined in FcγRIIB−/− mice from day 4 to day 22 after SRBC immunization. As shown in Fig. 9, there was no apparent difference in the kinetics of appearance or disappearance of total or different sized GL7− GCs in FcγRIIB−/− mice as compared with C57BL/6 mice (Fig. 1). These results suggest that while the development of the “mature” FDC reticulum is delayed, the general kinetics and magnitude of the GC B cell response remain unchanged due to absence of FcγRIIB.
Discussion

Within the microanatomic compartment of GCs, B cells undergo proliferation and V gene somatic hypermutation and selection accompanied by profound changes in the expression of a number of cell surface molecules such as IgD, CD38, and BLA-1 (27, 34). Our results demonstrate that the expression of FcγRIIB is also regulated in GC B cells. The levels of this receptor on GC B cells remain low at all stages of the response. Further, our data reveal that FDC "maturation" is perturbed in the absence of FcγRIIB, suggesting that this receptor may play an important role in activation or secondary development of FDCs. These observations have important implications for models proposed to account for the selection of high-affinity B cells in the GC.

Early models (13, 14, 35) explaining the mechanisms involved in memory B cell development proposed that B cells with somatically mutated Ag receptors are selected based on their capacity to be activated by Ag held as ICs on FDC. These models, however, did not consider the role of FcγRIIB. Later models that implicated a role for FcγRIIB in the selection of GC B cells were primarily based on data obtained from in vitro studies of primary B cells. Ravetch and Lanier (36) proposed that negative or positive selection of GC B cells depends on whether FcγRIIB alone is engaged or coengaged with the BCR, respectively. Interaction of ICs on FDC with B cells through FcγRIIB alone results in apoptosis, whereas coengagement with the BCR results in survival of B cells. Because our results demonstrate that FcγRIIB is substantially down-regulated on GC B cells, negative selection due to an apoptotic signal generated through FcγRIIB seems unlikely.

A model proposed by Tarlinton and Smith (37) suggested that early during the GC response, centrocytes usually initiate differentiation to Ab-forming cells (AFC). Ab produced by these AFC competes with centrocytes for the Ag present in the form of ICs on FDC in GC. Late in the primary response, when the Ab titer or affinity reaches a maximum, AFC differentiation diminishes, and a switch to memory B cell production takes place. This switch requires coengagement of the BCR and FcγRIIB. This model implies that FcγRIIB is not involved in the differentiation to AFC early in the GC response but is a requirement for memory formation later in the GC response. However, our data on the time course expression of FcγRIIB on GC B cells (Fig. 4) do not support this model. However, we did not examine whether FcγRIIB expression is reduced on GC B cells present in FDC-rich regions at all times during the GC response. Further studies will be required to determine whether a small subpopulation of GC B cells expresses normal levels of FcγRIIB at a specific time in the GC response.

In contrast to those described above, Tew et al. (38) proposed a model that takes into account the significance of FcγRIIB on FDC in selection of high-affinity GC B cells. According to this model, the high density of FcγRIIB on FDC allows them to bind most of the Fc regions in the ICs on their surface. This minimizes the interaction of these Fc regions with FcγRIIB on GC B cells, thus preventing the inhibition of B cell activity. The studies that led to the proposal of this model, however, did not examine the expression of FcγRIIB on GC B cells. Based on our findings, it appears that the primary mechanism by which GC B cells escape the inhibitory FcγRIIB signal is by down-regulating the expression of
this receptor. Our flow cytometric and RT-PCR analyses (Figs. 5 and 6), however, did reveal low level expression of FcγRIIB on GC B cells. Flow cytometric analysis performed on day 4 and later time points (day 16 and day 22) showed similar results (S. P. Rao and T. Manser, unpublished observations). Therefore, it is possible that both Fc blocking by FcγRIIB on FDC and the down-regulation of expression of FcγRIIB on B cells synergize to allow B cell stimulation by ICs. In any case, the functional relevance of such low FcγRIIB levels is not clear and will be the subject of future investigations.

Recent studies have suggested a role for FcγRIIB down-regulation in the development of autoimmunity. One study reported a 10-fold down-regulation on GC B cells in autoimmune-prone New Zealand Black mice and a 4-fold reduction on GC B cells in New Zealand White mice (17). In another study by the same group (18), FcγRIIB expression on the GC B cells from nonautoimmune mice, including C57BL/6, was up-regulated. The reasons for the discrepancy of our results and the results of this previous study are presently unclear. Factors that may account for this discrepancy are use of keyhole limpet hemocyanin as an immunogen and examining the expression of FcγRIIB on GC B cells after secondary challenge in their study. More likely, the differences are due to the criteria used for defining GC B cells. Although in our study FcγRIIB expression was analyzed on GC B cells that were defined as GL7⁺ IgD⁻, in the previous study all PNA⁺ cells were considered GC B cells. It was recently demonstrated that no single marker can unambiguously distinguish GC B cells from other subsets, and flow cytometric analyses showed that PNA⁺ cells include IgD⁻ cells (27). Therefore, analyses of surface expression of FcγRIIB on GC B cells defined on the basis of PNA binding alone may be misleading. These data emphasize the need to more thoroughly investigate the phenotypic criteria used in defining GC B cells.

It was also previously reported that FcγRIIB deficiency on a C57BL/6 background results in the development of severe autoimmune glomerulonephritis in old mice (39). These findings formed the basis for the suggestion (36) that absence or abnormal down-regulation of FcγRIIB is a mechanism that allows autoreactive IgG autoantibodies to be produced, predisposing to autoimmune disease. However, because our data show that down-regulation of FcγRIIB on GC B cells is characteristic of mice that are not autoimmune prone, the mechanistic role for FcγRIIB in the development of autoimmune conditions needs further evaluation. For example, there is no formal evidence demonstrating that autoreactive B cells in the GC, which in normal situations are believed to be negatively selected (13, 14), can be recruited into the memory pool due to down-regulation or absence of FcγRIIB.
Our data also provide new insights into the role of FcγRIIB in the "maturated" of FDC. We have reported that FDC-specific Abs, especially FDC-M1, recognize Ags that are induced on FDC in secondary but not primary follicles (16), suggesting that the expression of these Ags is associated with the maturation of FDC. Our data indicate that the gross GC response in FcγRIIB-deficient mice is comparable in kinetics and magnitude with that of normal mice. However, the time course histological analysis with FDC-specific Abs revealed a significant delay in the normally strong reticular expression pattern of the FDC-M1 and FDC-M2 Ags, indicating a defect in the timing of maturation of FDC in the GC. Moreover, during the early phases of the GC response in FcγRIIB-deficient mice, strong FDC-M1 staining colocalizes in a punctate pattern with MOMA-2 staining. Further studies are required to determine the nature of the cells that give rise to this staining pattern. They may be a subset of tingible body macrophages that express the FDC-M1 Ag, but it is also interesting to speculate that they are FDC precursors (40) that have yet to develop dendritic morphology.

B cell recall responses are thought to develop in GCs in response to ICs trapped on FDC. In this context, a recent study (16) investigated the significance of FcγRIIB on the accessory activity of FDC. In contrast to normal FDCs, those from FcγRIIB-deficient mice were incapable of augmenting IC-mediated B cell recall responses in vitro. Given our findings, it is possible that this lack of accessory activity of FDCs may be due not only to absence of FcγRIIB but to a defect in FDC maturation as well. More generally, our data suggest that the function of FcγRIIB on FDC is not merely to trap ICs but also to promote FDC maturation and expression of hitherto unknown molecules that may be involved in the regulation of GC B cell selection, memory development, and recall responses. Based on the correlation between the appearance of the FDC-M1 and FDC-M2 Ags and FcγRIIB up-regulation on FDC during immune responses, it is tempting to speculate that the pathways involved in the regulation of expression of these molecules are one and the same. Clearly, more studies are required to elucidate the role of FcγRIIB and mechanisms regulating the expression of this receptor on GC B cells and FDCs.

Another observation made in our study was the sectoring of GCs into two clearly discernible compartments when stained with Abs specific for FDC or FcγRIIB. This sectoring was observed at all times during the GC response and raises the possibility that these areas in murine GC may correspond to the light zone composed of centrocytes (area in which the FDC network is organized) and the dark zone composed of centroblasts (area where the FDC network is absent) reported in earlier studies on human tonsillar GCs (41, 42). If so, the delay in FDC maturation in FcγRIIB deficient mice may perturb the selection, affinity maturation and memory B cell development of centrocytes in the light zone. However, in the mouse, formal evidence that GC B cells in these areas actually represent centroblasts and centrocytes is currently lacking.

References


CORRECTIONS


*The Journal* received the following letter from Roderich E. Schwarz requesting correction of this article, which was published in the November 15, 1988 issue:

To the editor:

This is a formal request for a correction to a paper which appeared in *The Journal of Immunology* in November 1988 (R. E. Schwarz and J. C. Hiserodt), in response to a recommendation by the Office of Research Integrity (ORI) of the Public Health Service, Department of Health and Human Services. In 1994, the ORI had performed an investigation against the senior author of the manuscript titled “The expression and functional involvement of laminin-like molecules in non-MHC restricted cytotoxicity by human Leu-19+/CD3− natural killer lymphocytes,” the summary of which has been tagged to the PubMed reference of this article (http://grants1.nih.gov/grants/guide/notice-files/not94-105.html). In this ORI report, a requirement to correct the journal article, namely the indication that Fig. 2 of the article cannot be relied upon, was issued. Such recommendation remains sensible today, albeit 16 years after the original publication, as the investigation apparently failed to identify documented experimental data upon which the figure had been generated, and is hereby formally requested. However, the general ability to inhibit human adherent lymphokine-activated killer (A-LAK) cell cytotoxicity by F(ab′)2 of anti-laminin Ab, as stated in the legend of Fig. 2, should not be questioned. A figure, which is based on actual experimental data and reflects the inhibitory effect, is therefore added to this correction request, to replace the original Fig. 2.

**FIGURE 2.** Inhibition of cytotoxicity by two different human A-LAK cell populations through F(ab′)2 of affinity-purified anti-laminin Ab. Sorted Leu-19+/CD3− and Leu-19+/CD3+ A-LAK cell populations were mixed with Cr-labeled target cells in the continued presence of 150 μg/ml Ab for 4 h, and the resulting lytic activity was calculated as specified in Materials and Methods.
The Journal received the following letter requesting correction of this article, which was published in the August 15, 2002 issue:

The authors would like to alert the scientific community to the fact that we have been unable to reproduce one of the findings presented in this manuscript. In Fig. 5B of this manuscript we showed the results of flow cytometric studies designed to measure the levels of surface expression of FcγRIIB on splenic germinal center (GC) B cells (defined as B220⁺, IgD⁺, GL7⁺) as compared with splenic non-GC B cells (defined as B220⁺, IgD⁺, GL7⁻) using the anti-FcγRIIB mAb K9.361. These cells were isolated from C57BL/6 mice that had been immunized i.p. 8 days earlier with 3 × 10⁸ sheep RBC (SRBC) per mouse. Fig. 5B illustrated ~5-fold lower levels of K9.361 staining on GC B cells as compared with non-GC B cells. In Fig. 6, we presented the results of the semiquantitation of FcγRIIB mRNA levels, via RT-PCR and in gel hybridization, in these two populations of B cells that had been purified by FACS. This figure indicated ~6-fold lower levels of FcγRIIB mRNA in GC, as compared with non-GC B cells.

In multiple recent experiments designed to extend these published studies, neither the reduced levels of K9.361 surface staining of B220⁺, IgD⁺, GL7⁺ splenic B cells detected by flow cytometry or the reduced levels of FcγRIIB mRNA in such cells isolated by FACS 8 days after i.p. immunization of C57BL/6 mice with SRBCs (evaluated via real-time RT-PCR) have been observed.

In several other figures in the above-referenced manuscript, the results of immunohistological analysis of FcγRIIB expression in the GCs of SRBC immunized C57BL/6 mice were illustrated and interpreted to corroborate the results of the studies presented in Figs. 5 and 6. Due to the relative insensitivity of immunohistology as compared with flow cytometry, whether GC B cells stained 5- to 6-fold less intensely with anti-FcγRIIB mAbs as compared with non-GC B cells could not have been unequivocally determined using the former approach. Nonetheless, our previous interpretations of these immunohistological data with regard to levels of FcγRIIB on GC B cells appear to have been incorrect. In addition, arguments we forwarded in Discussion based on the conclusion that GC B cells express lower levels of FcγRIIB than non-GC B cells may no longer hold merit.

We currently can provide no compelling explanation for why our previously published results on the expression levels of FcγRIIB on GC B cells and the results of our more recent studies differ, but are actively investigating several possibilities. We should hasten to point out that our failure to reproduce the results presented in Figs. 5B and 6 does not influence the validity of any of the data or conclusions presented in the above-referenced manuscript regarding the expression and function of FcγRIIB on follicular dendritic cells.


The fifth author’s name, Emma J. O’Neill, was inadvertently omitted. The correct list of authors and affiliations is shown below.


The fifth author’s name, Emma J. O’Neill, was inadvertently omitted. The correct list of authors and affiliations is shown below.

Per O. Anderson, Anette Sundstedt, Zihni Yazici, Sophie Minaee, Richard Woolf, Kirsty Nicolson, Nathaniel Whitley, Li Li, Suling Li, David C. Wraith, and Ping Wang*

*Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, London, United Kingdom; †Tumor Immunology, Lund University, Lund, Sweden; ‡Department of Biological Sciences, Brunel University, Uxbridge, London, United Kingdom; and §Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol, United Kingdom

In Results, Fig. 2A1 was incorrectly published in black and white. The error has been corrected in the online version, which now differs from the print version as originally published. The correct color figure is shown below.

Also in Results, Fig. 3 fails to mention the headings “NFAT2” and “NF-κB (RelA)” on both panels a and b. The legend to this figure is written with the presumption that the headings are present on each of the two panels. The correct figure is shown below.


The second author’s last name is misspelled. The correct name is Stephen J. Pollock.
In the last summary of *In This Issue* titled “*Helicobacter pylori*-neutrophil interactions,” an error was made in citing the page number of the original article titled “*Helicobacter pylori* disrupts NADPH oxidase targeting in human neutrophils to induce extracellular superoxide release” by Lee-Ann H. Allen, Benjamin R. Beecher, Jeffrey T. Lynch, Olga V. Rohner, and Lara M. Wittine. The correct page number associated with the article is 3658. The error has been corrected in the online version, which now differs from the print version as originally published.


The sixth author’s first name is misspelled. The correct name is Chuangqi Chen.