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Fcγ Receptor IIB on Follicular Dendritic Cells Regulates the B Cell Recall Response

Dahui Qin,* Jiuhua Wu,* Kalpit A. Vora,† Jeffrey V. Ravetch,‡ Andras K. Szakal,§ Tim Manser,‡ and John G. Tew²*‡

Generation of the B cell recall response appears to involve interaction of Ag, in the form of an immune complex (IC) trapped on follicular dendritic cells (FDCs), with germinal center (GC) B cells. Thus, the expression of receptors on FDC and B cells that interact with ICs could be critical to the induction of an optimal recall response. FDCs in GCs, but not in primary follicles, express high levels of the IgG Fc receptor FcγRIIB. This regulated expression of FcγRIIB on FDC and its relation to recall Ab responses were examined both in vitro and in vivo. Trapping of IC in spleen and lymph nodes of FcγRII−/− mice was significantly reduced compared with that in wild-type controls. Addition of ICs to cultures of Ag-specific T and B cells elicited pronounced Ab responses only in the presence of FDCs. However, FDCs derived from FcγRIIB−/− mice supported only low level Ab production in this situation. Similarly, when FcγRII−/− mice were transplanted with wild-type Ag-specific T and B cells and challenged with specific Ag, the recall responses were significantly depressed compared with those of controls with wild-type FDC. These results substantiate the hypothesis that FcγRIIB expression on FDCs in GCs is important for FDCs to retain ICs and to mediate the conversion of ICs to a highly immunogenic form and for the generation of strong recall responses. The Journal of Immunology, 2000, 164: 6268–6275.

Memory is an intrinsic feature of the adaptive immune system, enabling the animal to mount a rapid and efficient recall response upon re-exposure to Ag. The generation of memory B cells has been proposed to be dependent on the ability of Ag to be trapped as immune complexes (ICs) in secondary follicles bearing germinal centers (GCs) (1–5). ICs form almost instantaneously in primed animals upon Ag challenge, and the vast majority is cleared by phagocytic cells (6, 7). However, some ICs are bound by a group of Ag transport cells that carry ICs into follicles where GCs develop. Follicular dendritic cells (FDCs) retain ICs on their surface, and convert some into IC-coated bodies (icososomes) (8, 9), which can be endocytosed by GC B cells. The icososomal Ag undergoes processing and presentation to T cells, which provide the help necessary for growth and differentiation (10, 11).

Dissection of this pathway in vitro yielded the finding that ICs are poorly immunogenic when added to Ag-specific B and T cell cultures, presumably due to the inhibitory role of FcγRIIB expres-

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mice resulted in animals that responded poorly to IC stimulation. These results suggest that FcγRIIB expression on FDCs is important for an optimal B cell recall response and provides an explanation for why ICs, despite their ability to mediate feedback regulation of B cell activity, are potent stimulators of the recall response in vivo.

Materials and Methods

Animals
Female C57BL or BALB/cByJ mice, 6–8 wk of age, were purchased from The Jackson Laboratory (Bar Harbor, ME). FcγRIIB knockout mice (25) were housed in standard plastic shoebox cages with filter tops. Food and water were supplied ad libitum, and the mice were used between 8–20 wk of age.

Aggs and immunization
Common FcγR chain −/−, FcγRIIB −/−, and +/+ mice (8–10 wk old, maintained on a mixed C57BL/6 × 129 background) were immunized i.p. (100 µg/mouse) with NP(4-hydroxy-3-nitrophenyl) acetyl)-chicken γ-globulin (CGG) precipitated in alum for induction of primary responses. For passive depositions of ICs, groups of mice were injected with rabbit anti-HRP (Sigma, St. Louis, MO) antiseraum containing 8 mg of Ig or the same amount of normal rabbit serum. One day later, the mice were injected with 11 µg of purified HRP (Sigma) i.p. in saline. The mice were killed 1 day later, and their spleens were frozen and sectioned as described below.

When OVA was used, wild-type C57 mice were primed by injecting 100–300 µg of OVA (Sigma catalogue no. A5503) precipitated with aluminum potassium sulfate (A7167, Sigma) in the nape of the neck as previously described (17, 26). Secondary immunizations were performed 2 wk later by injection into the front legs, hind feet, and i.p. (20 µg alum Ag/site).

Spleen sectioning and immunohistochemistry
Spleens were removed at various times after immunization and embedded in Tissue-Tek OCT compound (Fisher Scientific, Bridgewater, NJ) by flash-freezing in a 2-methylbutane bath cooled with liquid N2. Frozen spleens were stored at −80°C until sectioned. Six-micron-thick sections were cut on a cryostat microtome and thaw-mounted onto 0.05% poly-L-lysine (Sigma)-coated slides. Sections were allowed to air-dry, then were fixed in ice-cold acetone for 10 min, air-dried, and stored at −80°C. The frozen sections were thawed and rehydrated in PBS for 20 min. Endogenous peroxidase activity was blocked by immersing the sections in 0.3% (v/v) aqueous H2O2 solution. The sections were then blocked with 5% BSA and 0.1% Tween-20 in PBS. They were labeled with mAbs 8C12-biotin (anti-CRI, Pharmingen, San Diego, CA), 2.4G2 (anti-FcRII/III, Pharmingen), and FDC-M1 or FDC-M2 (anti-follicular dendritic cell, gifts from Dr. Marie Kosco-Vilbois, Serono Pharmaceutical Research Institute, Plan-les-Ouates, Switzerland) Abs. Slides labeled with 2.4G2, FDC-M1, and FDC-M2 were further developed using alkaline phosphatase (AP)-conju- gated F(ab′)2 mouse anti-rat Ig (Jackson Immunoresearch Laboratories, West Grove, PA). All slides labeled with biotinylated Abs were then labeled with streptavidin-AP (Southern Biotechnology Associates) and AP-labeled streptavidin (Kirkegaard & Perry, Gaithersburg, MD). The levels of anti-OVA in the cultures were determined from standard curves prepared using a standard serum in each ELISA assay. This standard anti-OVA serum was collected from hyper-immunized BALB/c mice, and the anti-OVA level in serum was determined using quantitative precipitin analysis (31).

FDC isolation
FDCs were isolated from the lymph nodes (popliteal, brachial, axillary, inguinal, periaortic, and mesenteric) using previously described procedures, except higher levels of irradiation were used (17). The high irradiation doses did not interfere with FDC functions; this may be due to a high level of thiol compounds in FDCs (27), which can protect against radiation injury. Three days after irradiation, the lymph nodes were removed from the mice and cut with 26.5-gauge sterile needles to facilitate enzymatic digestion. The cut nodes were incubated with 1 ml of 8 mg/ml collagenase D (lot FIA148, Roche, Indianapolis, IN) and 0.5 ml of 10 mg/ml DNase I (lot 32H9545, Sigma) in 1 ml of complete DMEM at 37°C. After 1-h incubation, cells were released from the stroma by gentle pipetting, and the media containing the free cells were collected. The isolated cells were then directly layered onto a continuous 50% Percoll gradient and centrifuged for 20 min at 700 × g. The low density (1.050–1.060 g/ml) FDC-enriched fraction was then removed and washed twice. Finally, the washed cells were incubated in petri dishes at 37°C for 1 h to remove adherent macrophages. The nonadherent cell suspension typically contained 30–50% FDCs as determined by flow cytometry using the FDC-specific mAb FDC-M1. The vast majority of the contaminating cells were medium to large lymphocytes.

FDC depletion
FDCs were depleted from enriched FDC preparations using biotin-la- beled FDC-specific mAb, FDC-M1 (26), as described previously (28). In brief, FDC preparations were incubated with rat anti-mouse FcγR Ab (2.4G2) at 4°C for 30 min to block nonspecific Fc binding of rat mAb. Biotinylated FDC-M1 was then added and incubated with the cells for 30 min. After washing the cell fraction three times, streptavidin-co- valently coupled magnetic Dynabeads (M-280, lot 3171, Dynal, Oslo, Norway) were added at a concentration of 15 beads/target cell in a final volume of 500 µl. After a 30-min incubation at room temperature on a shaking platform, FDCs bound to Dynabeads were separated by a Mag- netic Particle Concentrator (Dynal, Great Neck, NY). This step removes about 90% of the FDCs, leaving an FDC-depleted fraction (28).

FACS analysis
FDCs were isolated from immunized C57 mice as described above. The FDC preparation was split into two aliquots and incubated with FITC-conjugated 2.4G2 Ab (PharMingen) for 30 min in the cold. The aliquots of FDCs were then incubated with biotinylated FDC-M1 or biotinylated iso- type control IgG for 2h in the cold. After washing twice, the cells were incubated with streptavidin-conjugated PE for 30 min in the cold. The labeled cells were observed using FACSscan. Data for 10,000 cells from each aliquot were collected and analyzed.

Lymphocyte preparation
Memory lymphocytes were obtained from draining lymph nodes of OVA-immunized mice 1 mo or more after the final OVA challenge. The lymph nodes were bathed in complete DMEM with 10% FBS and ground between two sterile slides. This harsh treatment of the lymph nodes disrupts Ag- bearing FDCs and plasma cells (29). Consequently, very little Ab is produced when these cells are cultured in the absence of added Ag. After disrupting the lymph node and releasing the cells with the slides, the cell suspensions were filtered through nylon mesh to remove stromal tissue.

Cell cultures
Enriched FDC preparations (1 × 105 cells) were added to 3 × 105 B and T memory cells in 96-well tissue culture plates (catalogue no. 3595, Costar, Cambridge, MA) containing 200 µl of complete culture medium/well. The culture medium used in all studies consisted of DMEM supplemented with 10% FCS, 20 mM HEPES, 2 mM glutamine, 50 µg/ml gentamicin, and MEM-nonessential amino acids. The cell cultures were incubated at 37°C in a 5% CO2 incubator for 14 days, and medium was harvested on days 7 and 14.

Ab assay
Culture medium was collected from the lymphocyte cultures every 6–7 days and anti-OVA-specific IgG was measured by means of a solid phase ELISA as described previously (30). Murine IgG specifically bound to OVA was detected using biotinylated goat anti-mouse IgG (Southern Bio- technology Associates) and AP-labeled streptavidin (Kirkegaard & Perry, Gaithersburg, MD). The levels of anti-OVA in the cultures were deter- mined from standard curves prepared using a standard serum in each ELISA assay. This standard anti-OVA serum was collected from hyper- immunized BALB/c mice, and the anti-OVA level in serum was deter- mined using quantitative precipitin analysis (31).

In vivo IC trapping
The mice used in these studies were given water containing KI (50 µg/ml) for 1 wk to saturate iodine in the thyroid gland. The serum used to from IC was obtained from hyperimmunized mice (primed and boosted twice) contain- ing 1.5 mg/ml anti-OVA IgG. The iodinated HSA-anti-HSA IC was injected s.c. into the feet on the right site (2.5 µg/site). Fourteen days later spleen and draining and nondraining popliteal lymph nodes were harvested. Macrophages trapped, but rapidly degraded, IC made of [125 I]HSA-anti-HSA IC was injected s.c. into the feet on the right site (2.5 µg/site). Forty days later spleen and draining and nondraining popliteal lymph nodes were harvested. Macrophages trapped, but rapidly degraded, IC made of [125 I]HSA-anti-HSA IC was injected s.c. into the feet on the right site (2.5 µg/site). Forty days later
small amount of the iodinated HSA was saved and counted at the same time as the lymph nodes and spleens to convert the counts to picograms of retained Ag (21, 32).

Results

FcyRIIB expression on FDCs and up-regulation in secondary follicles

To determine which of the Fcγ receptors is expressed on FDCs, mice lacking expression of FcγRI and FcγRIII due to the absence of the common FcR γ-chain were exploited. In sections FDCs appear as integral members of a sponge-like network known as the follicular reticulum or FDC-reticulum. These FDC-reticula are formed by interdigitating dendrites from a number of FDCs. Spaces within this spongework of FDCs are filled with B cells and some T cells. This microenvironment, located in the light zone of secondary follicles, brings together Ag, FDCs, B cells, and Th cells. Spleen sections obtained from immunized γ/− mice (that lack FcγRI and FcγRIII due to the absence of the common FcR γ-chain) were labeled with the anti-CR1 mAb 8C12 or the mAb 2.4G2, which detects both FcγRI and FcγRIII. As shown in Fig. 1, the follicular reticulum was easily visualized by anti-CR1 (A) as previously reported (33). In reactive follicles containing GCs (C, arrows) FDCs could be visualized using the mAbs FDC-M2 (B) and FDC-M1 (C). These same regions of the reactive follicles labeled strongly with 2.4G2 (D), which in the γ/− background only detected FcyRIIB. Importantly, identical labeling results were obtained using immunized wild-type mice (data not shown).

To confirm that FDC-M1 and 2.4G2 were labeling the same cells, we used a two-color histological analysis of Fcγ-reticula (Fig. 2). Adjacent parallel sections were labeled using 2.4G2 (Fig. 2A, red labeling), FDC-M1 (Fig. 2B, green labeling), and then both labels on the same section (Fig. 2C). The overlapping of the green and red gave the yellow Fcγ-reticulum shown in Fig. 2C. The double labeling was further established by testing FDC in a single-cell suspension (confirmed by microscopy) using immunized mice as donors and staining using FDC-M1 and 2.4G2. Nearly 30% of the cells in the FDC-enriched preparation labeled with FDC-M1 and FACS analysis revealed that almost all these cells also labeled with 2.4G2.

In contrast with Fig. 1, Fig. 3 presents representative results from a similar histological analysis of the spleens of mice with a targeted inactivation of the FcγRIIB gene. While the A panels show that the primary follicular reticulum as defined by 8C12 labeling is well developed in FcγRIIB−− spleen, 2.4G2 labeling of the follicular reticulum (C panels) is absent in GCs (arrows), and FDC-M1 labeling (B panels) is reduced. Collectively, these data define the FcγR on FDCs in GCs as FcγRIIB, consistent with previous suggestions (23, 34), and indicate that the level of expression of this receptor is highly induced on FDCs in this microenvironment.

To further substantiate the latter point, an identical histological analysis was performed on spleens obtained from young unimmunized wild-type mice housed in specific pathogen-free conditions. The spleens of such mice contained mainly primary follicles lacking GCs. Fig. 4 presents representative data from this experiment. The primary follicular reticulum is easily visualized with 8C12 labeling (A), but reticular labeling in the primary follicle is not observed using FDC-M1 or 2.4G2.

Absence of FcyRIIB reduces immune complex trapping in the follicle

While passively formed ICs strongly label the splenic follicular reticulum in wild-type mice (Fig. 5A), these same ICs give rise to reduced labeling of this reticulum in FcγRIIB−− mice (Fig. 5B). A semiquantitative analysis of IC deposition in these splenic sections indicated that IC trapping was reduced severalfold in the FcγRIIB−− mice. These results were confirmed and extended using radiolabeled ICs to quantitate the amount of IC trapping in both splenic and lymph node (LN) follicles (Table I). Retained IC persisted in the spleen and draining LNs, in contrast to nondraining LNs. The amount of Ag retained in FcγRIIB−− mice was reduced to about 25% of normal in the spleen, which is compatible with the histochemistry, and to 50% of normal in the draining LNs.

FcyRIIB in the conversion of IC into potent immunogens

To directly test the hypothesis that FDCs are able to convert a poorly immunogenic IC into a highly immunogenic form, OVA-containing ICs were added to OVA-primed T and B cells derived
from normal mice, and the level of secretion of OVA-specific IgG Ab was measured. In the absence of FDCs only picogram levels of anti-OVA were induced at any dose of IC used (Fig. 6A). In contrast, addition of FDCs from normal mice to these cultures elicited substantial levels of OVA-specific IgG over a wide dose range of IC.

Because the FDC preparation used in Fig. 6 was enriched but not pure, depletion studies were conducted to exclude the possibility that the adjuvant-like activity was mediated by other cells. The FDC-specific Ab FDC-M1 was used to generate an FDC-depleted fraction. As shown in Fig. 7B, depletion of most FDCs resulted in a 90% reduction of the stimulatory response observed, confirming that FDCs in the in vitro culture of memory T and B cells were responsible for the stimulatory effect of IC. Because the data presented above show that expression of the FDC-M1 epitope and FcγRIIB on FDCs is highly correlated, FDCs expressing high levels of FcγRIIB would have been preferentially depleted in this experiment.

In vivo, FDCs may engage ICs through FcγRIIB, CR1/2, or a combination of these receptors. To determine whether the FcγRIIB on FDCs has the ability to convert the IC to a potent immunogen in vitro, FDCs were prepared from FcγRIIB1/1 or FcγRIIB2/2 mice and added to cultures of OVA-primed T and B cells. Deficiency of FcγRIIB on the FDCs dramatically reduced the stimulatory effect of these cells on IgG Ab production (Fig. 7A), establishing that this receptor is important for enabling the FDCs to convert the IC into a potent immunogen in vitro. However, the addition of FDC from the FcγRIIB2/2 mice to the memory cells plus OVA-anti-OVA increased anti-OVA production from <10
ng/ml to >500 ng/ml, indicating that some adjuvant activity persisted in the absence of FcγRIIB. Because the development or regulation of FDC activity may be perturbed in FcγRIIB−/− mice (see below), analogous experiments were performed by blocking FcγRIIB (with soluble 2.4G2) in cultures containing FDCs derived from wild-type mice. Results similar to those achieved with FDCs isolated from FcγRIIB-deficient mice were obtained (Fig. 7B).

These in vitro results suggested that FcγRIIB mediate a specific function on FDCs by enabling ICs to stimulate a B cell recall response in vivo. To dissect the in vivo role of FcγRIIB on FDCs, LN lymphocytes were obtained from OVA-immune mice and were injected into irradiated wild-type and FcγRIIB−/− mice. Twenty-four hours after adoptive transfer, mice in both groups were injected i.v. with preformed OVA-anti-OVA ICs. Preformed ICs were used because there was no specific anti-OVA in the recipients to convert OVA into ICs as there would be in typical recall responses. Two weeks later the mice were bled, and the levels of serum anti-OVA IgG generated during this recall response were determined. ICs generated a potent serum recall response in wild-type recipients (Fig. 8), while the response in FcγRIIB−/− recipients was significantly diminished. To determine whether an additional immunization would overcome the reduced response of the wild-type lymphocytes in FcγRIIB−/− mice, both groups of mice were challenged with free OVA. Two weeks later, serum Ab levels were determined and compared. The specific anti-OVA levels in wild-type mice increased to >2 mg/ml (a robust response), while the response in FcγRIIB−/− mice remained low (<10% of that level; p < 0.01). These in vivo results support the in vitro studies presented above and suggest that FcγRIIB functions specifically to enable the retained IC to stimulate B cells in the secondary follicular reaction.

Discussion

Ag in the form of ICs has been proposed to be essential to the formation of the GC (2, 5) and the generation of the secondary response (2, 17). Two classes of molecules expressed in the follicle have the capacity to bind ICs: the complement receptors for C3d

FIGURE 4. FcγRIIB and the epitope recognized by FDC-M1 are not expressed on the follicular-reticula of primary follicles. Parallel sections of the spleen of a young, unimmunized C57BL/6 mouse were labeled with anti-CR1 (A), FDC-M1 (B), and 2.4G2 (C) Abs (all blue) and counterstained with peanut agglutinin (red, only background labeling was obtained in the absence of germinal centers). Note that the follicular-reticula was labeled with anti-CR1 in A. In contrast, the follicular-reticula were not labeled in parallel sections with FDC-M1 and 2.4G2. (The light labeling with 2.4G2 in this region is of an intensity consistent with B cell FcγRIIB expression.) This is in marked contrast to the reticula in GC shown in Figs. 1 and 3, which label brightly with FDC-M1 and 2.4G2. B: The red pulp (RP) and white pulp (WP) regions are labeled. Original magnification, ×100.

FIGURE 5. Follicular trapping of ICs by FDCs in FcγRIIB−/− mice is reduced compared with that in FcγRIIB+/+ littermates. The two groups of mice were passively immunized with HRP-anti-HRP ICs as described in Materials and Methods. Spleens were taken 3 days later and processed for immunohistochemistry as described previously (33, 48). This follicular-reticulum was labeled using the anti-CR1 mAb 8C12 (blue), and the trapped HRP containing ICs are labeled red. Note the reduced IC trapping in FcγRIIB−/− reticulum (B) compared with the heterozygous control (A). This figure is representative of sections prepared from three FcγRIIB−/− mice that were compared with sections from three FcγRIIB+/+ controls. Analysis of the HRP deposition in these splenic sections indicated that IC trapping was reduced severalfold in the FcγRIIB−/− mice. Original magnification, ×400.
CR1/2-deficient mouse is sufficient to reconstitute a substantial (41). Nevertheless, transfer of CR1/2-expressing B cells into a reported that CR1/2 on FDC is important for a strong Ab response may also play a role in an immune response. For example, it is that have entered the GC provided by a ligand present on the FDC, CD19 complex (38) and by delivering a survival signal to B cells survival within the GC (36, 37). These receptors play a positive role CR1/2 on B cells is essential for their stimulation as well as sur-

tion on each cell type is apparently quite different. Expression of (22, 23, 35). These receptors are expressed on both B cells and

and C4b (CR1/2) (18 –20) and the Fc receptors for IgG ICs (Fc R) (22, 23, 35). These receptors are expressed on both B cells and FDCs; however, given our results and those of others, their function on each cell type is apparently quite different. Expression of CR1/2 on B cells is essential for their stimulation as well as sur-

vival within the GC (36, 37). These receptors play a positive role in mediating B cell stimulation through their interaction with the CD19 complex (38) and by delivering a survival signal to B cells that have entered the GC provided by a ligand present on the FDC, presumably C3d (36, 39, 40). The expression of CR1/2 on FDCs may also play a role in an immune response. For example, it is reported that CR1/2 on FDC is important for a strong Ab response (41). Nevertheless, transfer of CR1/2-expressing B cells into a CR1/2-deficient mouse is sufficient to reconstitute a substantial immune response and leads to the generation of memory cells (36, 37, 42).

FcγRIIB expression on B cells can result in the generation of an inhibitory signal, triggered by its coligation to the BCR by ICs (12–16). Given the general inhibitory function of FcγRIIB on he-

mopoietic cells (12–16), it was unexpected that the capacity of the FDCs to convert ICs into a form capable of stimulating B cell activation was dramatically enhanced by FcγRIIB expression on FDC. Strikingly, our results indicate that FcγRIIB functions as a positive regulator of the adjuvant property of FDCs. Interestingly, a balanced effect was obtained in FcγRIIB−/− mice, where B cell activation and proliferation would not be down-regulated by FcγRIIB, but trapping of Ag by FDCs was substantially reduced (Fig. 5 and Table I). The net outcome of the opposing effects was minimal, as the immune response in the FcγRIIB−/− mice was essentially normal (25). This normal response stands in marked contrast with the subnormal recall response obtained in the present study when wild-type B memory cells bearing FcγRIIB were used in combination with FDC from FcγRIIB knockout mice (Figs. 7A and 8). These observations suggest that FcγRIIB-bearing FDC are critical for recall responses derived from wild-type FcγRIIB-bearing B cells, and this concept is consistent with results from models where the lack of FDC is associated with a lack of germinal centers and the recall Igs IgG and IgA (43–45). FDC deficiencies occur in animals lacking lymphotoxin or TNF, or receptors for these cyto-

kines (33, 43).

Table 1. Trapping of IC in spleen and LN of FcγRII−/− and FcγRII−/− mice

<table>
<thead>
<tr>
<th>Organs</th>
<th>Wild Type (pg of HSA)</th>
<th>Knockout (pg of HSA)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>449.0 ± 58.0^b</td>
<td>127.0 ± 41.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Spleen/mg</td>
<td>9.1 ± 1.7</td>
<td>2.1 ± 0.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Draining PLN</td>
<td>158 ± 9.9</td>
<td>83.7 ± 1.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Draining PLN/mg</td>
<td>73.9 ± 7.6</td>
<td>37.3 ± 7.8</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Nondraining PLN/mg</td>
<td>2.09 ± 0.5</td>
<td>2.03 ± 0.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE from seven FcγRII−/− mice and five FcγRII−/− mice.

FIGURE 6. Ability of FDCs to convert poorly immunogenic IC into an effective immunogen for secondary responses in vitro. A, Memory T and B lymphocytes (Ly) were cultured with various amounts of OVA-anti-OVA IC formed near equivalence in the presence or the absence of FDCs from normal mice (5 × 10^5 Ly/well with or without 1 × 10^5 FDC). B, FDCs from the LN of normal mice or the same number of the cells contaminating the FDC preparation (after depleting FDC) were added to memory T and B cells taken from OVA-immune mice. Ten nanograms of OVA in an OVA-anti-OVA IC near equivalence was used as the immunogen. Cultures were washed on day 7 to remove any free IC, and anti-OVA was measured on day 14. Controls included normal memory T and B lymphocytes cultured alone, T and B lymphocytes plus IC, and FDCs alone, and all produced <10 ng of anti-OVA/ml (data not shown). These data are typical of three experiments of this type.
The molecular mechanisms used by FDCs to amplify IC B cell differentiation, or Ab production, this model would suggest that when stimulated B cells interact with FDCs in the absence of ICs they receive survival signals (28, 36), but not differentiation signals (47). However, after the GC becomes well developed, the induction and engagement of FcγRIIB on FDCs allow them to participate in the regulation of B cell isotype class switching and differentiation to Ab-forming cells. It is intriguing to speculate that FcγRIIB engagement on FDCs induces the generation of IC-containing iccosomes that, once internalized by follicular B cells, would provide the intracellular levels of cognate Ag necessary for MHC class II-mediated presentation and receipt of T cell help.

The selective and up-regulated expression of FcγRIIB on FDCs in reactive follicles containing GCs further supports the idea that ICs play an essential role in the B cell recall response (2, 17). During this phase of the secondary immune response it is critical that B cells are appropriately activated, and Ab-forming cells and memory cells are formed. After a productive secondary response it may be important to down-regulate FcγRIIB expression on FDCs and thereby facilitate termination of the GC reaction by allowing more IC to bind to the B cell FcγRIIB and trigger inhibition. The results presented here support a context regulation model of the secondary response in which FcγRIIB expression on FDCs influences whether an IC is stimulatory or inhibitory to a B cell.

References


FIGURE 8. Comparison of anti-OVA IgG production in FcγRIIB+/+ and FcγRIIB−/− mice adoptively transplanted with leukocytes from wild-type OVA-immune mice. FcγRIIB+/+ or FcγRIIB−/− mice were irradiated with 600 rad and reconstituted with 2×107 leukocytes obtained from the LN of OVA-immune wild-type C57BL/6 mice. One day after reconstitution, the mice were challenged i.v. using preformed OVA-anti-OVA immune complex (5 μg of Ag-containing IC/mouse). The serum anti-OVA IgG titers were measured 14 days after the IC challenge, using ELISA. These mice were given a booster immunization using free Ag (5 μg of OVA/mouse i.p.) at 3 wk, and 2 wk later serum anti-OVA IgG titers were again determined. Five mice were used in each group, and the results are the mean ± SE.