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Immune Complex and Fc Receptor-Mediated Augmentation of Antigen Presentation for in Vivo Th Cell Responses

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It has recently been established that FcRs are involved in the triggering of type II and III inflammatory responses. Although FcR is not believed to be involved in the regulation of T cell function, the in vivo contribution of FcRs to T cell function still remains unclear. We analyzed in vivo responses of delayed-type hypersensitivity and proliferation of CD4+ T cells to Ags in FcRγ−/− mice lacking the expression and function of FcγRI, FcγRII, and FcγRII. We found that the delayed-type hypersensitivity response in FcRγ−/− mice is significantly decreased compared with that in wild-type mice. Moreover, the secondary responses of proliferation and cytokine production as well as the Ab formation by CD4+ T cells from FcRγ−/− mice to Ag and normal APCs were also reduced. In contrast, in vitro primary T cell proliferative responses upon stimulation with anti-TCR Ab or MLR as well as in vivo primary responses against staphylococcus enterotoxin B administration were not different between T cells from FcRγ−/− and wild-type mice. In addition, the Ag presentation function of APCs from unimmunized FcRγ−/− mice was normal. On the other hand, Ab-deficient mice also revealed impaired T cell responses. These results demonstrate that the defective T cell responses in FcRγ−/− mice were due to impaired Ag presentation during in vivo priming not to a defect in T cells. Therefore, they suggest that the FcRs on APCs mediate efficient priming of Th cell responses in vivo in an immune complex-dependent manner. The Journal of Immunology, 2000, 164: 6113–6119.

immune complexes activate various FcR-expressing cells, and FcRs play important roles in triggering various autoimmune and inflammatory diseases. FcRs play an important role in the connection between humoral and cellular responses (1, 2). In the mouse system, there are three types of FcγRs: a high affinity receptor, FcγRI (CD64), which is capable of high affinity-binding to monomeric IgG; and two low affinity receptors, FcγRII (CD32) and FcγRIII (CD16), which exhibit low affinity-binding to monomeric IgG and then bind mainly to polymeric IgG. FcγRI and FcγRIII are composed of multimeric subunits and require a homodimer of the γ subunit (FcγRI) for their assembly and cell surface expression as well as for signal transduction through FcγR. The FcγR chain is also associated with the high affinity FcR complex for IgE (FceRI).

Previous work demonstrated that FcγRγ-deficient (−/−) mice showed no expression of FcγRI, FcγRII, and FcγRII on the surfaces of various cells (3, 4). In addition, IgE-mediated anaphylaxis by mast cells, which is a representative type I inflammation, is impaired in Fcγγ−/− mice (3, 5). More importantly, it was shown that Arthus reaction induced by immune complex (IC)3 of specific Ag and Ab, a representative type III inflammation, was severely reduced in both Fcγγ−/− and FcγRIII-deficient mice (3, 6). Furthermore, Fcγγ−/− mice failed to induce IgG-mediated phagocytosis by macrophages, and they also exhibited severe reduction in the induction of autoantibody-dependent experimental hemolytic anemia and thrombocytopenia (7), anti-glomerular basement membrane Ab-induced glomerulonephritis (4), and IC-induced vasculitis syndrome (34). These results indicated that FcγRs play important roles in not only type I but also type II and III inflammatory responses. However, it still remains unclear whether FcRs is also involved in a type IV inflammation. Regarding the type IV response, the effects of the functions of FcRs on even normal T cell functions have not been analyzed except for FcγR-expressing intestinal intraepithelial T cells (8) and NK1.1+ T cells (9).

Dendritic cells (DCs) and macrophages are the major APCs in the immune system and are involved in the activation and differentiation of CD4+ and CD8+ T cells (10, 11). Previously, it was reported that Ags internalized through specific membrane receptors such as surface Ig and FcRs are more efficiently presented to CD4+ T cells compared with those internalized in the fluid phase in the case of MHC class II-restricted presentation (12). Especially, it is well known that FcRs are responsible for internalization of Ag by forming ICs with specific Ab and facilitating efficient MHC class II-restricted presentation in vitro. Similarly, FcγRs also promote efficient MHC class I-restricted presentation of peptides by internalization of exogenous Ag-IgG ICs (13). FcγRI and FcγRIII trigger activation signals through FcγR, which bear an immunoreceptor tyrosine-based activation motif (1, 14). Indeed, signals from FcγRs cause the maturation of DCs (13) and promote efficient MHC class I and II-restricted Ag presentation. However, the in vivo function of FcRs in MHC class I and II-restricted Ag presentation to T cells is still poorly understood.

To clarify the role of FcR in type IV inflammatory responses and MHC class II-restricted Ag presentation to CD4+ T cells in vivo, we analyzed delayed-type hypersensitivity (DTH) and proliferative responses of CD4+ T cells from Fcγγ−/− mice upon stimulation with specific Ags. We found that the priming of CD4+ T cells with a specific Ag is significantly impaired in Fcγγ−/− mice.

1 Abbreviations used in this paper: IC, immune complex; DC, dendritic cell; DTH, delayed-type hypersensitivity; KLH, keyhole lampet hemocyanin; PPD, purified protein derivative; SEB, staphylococcus enterotoxin B; LN, lymph node; SAg, superantigen; μMT, membrane IgM-deficient mice.

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mice in spite of the fact that the function of T cells from unimmunized mice was normal. Together with the observation that the impaired T cell responses were also observed in Ig-deficient mice, these observations suggest that FcRs expressed on APCs play an important role in the priming of Th cell responses in vivo.

Materials and Methods

Mice

C57BL/6 and BALB/c mice were purchased from the Shizuoka Animal Center (Hamamatsu, Japan). The establishment and characterizations of FcγR−/− mice with C57BL/6 background have been described previously (4). Membrane IgM-deficient mice (μMT) mice were produced as described previously (15) and were provided by Dr. D. Kitamura (Tokyo Science University, Chiba, Japan). All mice were bred and maintained in our own animal facility under specific pathogen-free conditions.

Delayed-type hypersensitivity

To measure DTH responses, we examined the swelling responses of footpads in mice. The basic protocol of the swelling assay was described previously (16). Briefly, mice were immunized by s.c. injecting 200 μl emulsion of CFA containing 200 μg of KLH at three sites, one on each flank and the third at the base of the tail. Two weeks or 10 wk after sensitization, 100 μg of keyhole limpet hemocyanin (KLH) in 20 μl of PBS was injected into the right footpad, and 20 μl of PBS was injected into the left footpad as a control. After 24 h, footpad swelling was measured (in mm) with a micrometer as [(right footpad thickness after challenge − right footpad thickness before challenge) − (left footpad thickness after challenge − left footpad thickness before challenge)].

Proliferative response of T cells

Mice were immunized with 200 μg of KLH or OVA with CFA intradermally in both flanks and at the base of the tail for analysis of splenocytes. Similarly, mice were immunized with 200 μg of KLH with CFA intraderrmally in both fore and hind footpads for analysis of lymph node cells. Three each of spleen or popliteal and axillary draining lymph nodes from immunized mice were obtained 1, 2, 4, or 6 wk after immunization. Splenocytes and lymph node cells were mixed with anti-CD8 mAb (53.6.7) and incubated with magnet beads (Advanced Magnetics, Cambridge, MA) coupled with goat anti-mouse IgG Ab and goat anti-rat IgG Ab to remove surface Ig+ B cells and CD8+ T cells. For the proliferative response of splenocytes in wild-type, FcγR−/−, and μMT mice, CD4+ T cells were purified by using MACS (Miltenyi Biotec, Bergisch Gladbach, Germany) 3 wk after immunization. The respective purities were 94, 96, and 93%. The cells were cultured in 96-well U-bottom microplates (Falcon; Becton Dickinson, Mountain View, CA) in the absence or presence of graded concentrations of KLH, purified protein derivative (PPD), or OVA at 37°C in 5% CO2 for 4 days, pulsed with 0.5 μCi of [3H]thymidine for 8 h, and harvested on glass filters. The incorporated radioactivity was measured with a MicroBeta liquid scintillation counter (Wallac, Gaithersburg, MD). The results of the [3H]thymidine incorporation assay were expressed as the mean cpm ± SD of triplicate cultures from each group of mice.

Titration of anti-KLH Abs in serum

Serum Ab titers were measured by modification of an ELISA assay. A 96-well microplate was coated with 50 μl/well of a 50 μg/ml solution of KLH in 0.1 M NaHCO3, at 4°C overnight and then was blocked with 50 μl/well of PBS containing 3% BSA at room temperature for 3 h. Serum was added at 50 μl/well and allowed to react at room temperature for 3 h. The wells were washed five times with PBS containing 0.05% Tween 20 and incubated with 50 μl of anti-mouse IgM, IgG1, IgG2a, IgG2b, or IgG3 coupled to HRP at room temperature for 1 h. Thereafter, plates were washed six times with PBS containing 0.05% Tween 20 before incubation at room temperature for 20 min with o-phenylenediamine as a substrate (Wako, Osaka, Japan).

Measurement of cytokine production

Culture supernatants were removed from wells after 72 h of culture, and the concentrations of IL-2, IL-4, and IFN-γ were measured by ELISA by standard protocol as described previously (17). For analyzing the function of Ag presentation, IL-2 production from a T cell hybridoma was measured. An OVA-specific murine T cell hybridoma, DO11.10, was cultured with irradiated splenocytes from wild-type and FcγR−/− mice with BALB/c background in the presence of various concentrations of OVA peptide 323–339 and soluble OVA (18). Culture supernatants were collected from wells after 24 h of culture, and the concentration of IL-2 was measured by ELISA and used as the levels of cytokines, and serum levels of Abs were calculated using Student’s t test or Welch’s t test. A p value <0.05 was considered significant.

Results

Defective responses of DTH and T cell proliferation in FcγR−/− mice

We first immunized wild-type and FcγR−/− mice s.c. with KLH in CFA, and footpad swelling was measured upon challenging with Ag at 2 and 10 wk after immunization to examine the contribution of FcRs in the DTH response. As shown in Fig. 1, the DTH response in FcγR−/− mice was very low compared with that in wild-type mice regardless of the sensitization period, clearly indicating that FcγR is involved in DTH response.

Then, to examine whether the defect in the DTH response was due to a functional difference of T cells in FcγR−/− and wild-type mice after immunization with Ag, we analyzed the in vitro
FIGURE 2. Ag-specific proliferative responses of CD4+ T cells from FcRγ−/− mice (●) and wild-type mice (○). A and B, Proliferative responses of splenic T cells to KLH. Four weeks after immunization with KLH in CFA, splenic CD4+ T cells (3 × 10^5/well) were purified and stimulated in vitro with various concentrations of KLH (A) and PPD (B) for 4 days, and proliferative responses were measured. Data are representative of nine independent experiments. C, Proliferative responses of splenic T cells to OVA. Four weeks after immunization, purified splenocytes (3 × 10^5/well) were stimulated with OVA for 4 days. Data are representative of two independent experiments. D, Proliferative responses of LN T cells to KLH. Six weeks after immunization, purified draining LN cells (3 × 10^5/well) were stimulated with KLH for 4 days. Data are the representative of three independent experiments. The results are shown as the mean of three mice ± SD. **, p < 0.01; *, p < 0.05; compared with wild-type mice.

Effect of duration and Ag dose of priming on suppression of T cell response in FcRγ−/− mice

We compared the effect of the time course of priming of CD4+ T cells on the proliferative responses between wild-type and FcRγ−/− mice. Splenocytes were obtained from mice 1, 2, and 4 wk after immunization with KLH, and CD4+ splenetic T cells were stimulated in vitro with KLH in the presence of wild-type APCs secondary proliferative responses of purified CD4+ T cells from immunized FcRγ−/− and wild-type mice upon stimulation with the Ag and irradiated splenocytes from wild-type mice. As shown in Fig. 2, A and B, proliferative responses of CD4+ T cells from KLH-immunized FcRγ−/− mice were very low upon stimulation with both KLH and PPD in comparison with those from wild-type mice. The response to PPD was raised due to the use of CFA for immunization. Similar results were obtained when OVA was used as Ag (Fig. 2C). Reduced proliferation of CD4+ T cells from FcRγ−/− mice was also observed upon immunization with KLH inIFA (data not shown). Furthermore, CD4+ T cells from draining lymph nodes (LN) of FcRγ−/− mice also exhibited low proliferation upon immunization with KLH in CFA (Fig. 2D).

FIGURE 3. Effect of duration and Ag dose of priming on suppression of T cell proliferation and cytokine production in FcRγ−/− mice (●) and wild-type (WT) mice (○). A, Effect of duration of Ag priming. Splenic CD4+ T cells (3 × 10^5/well) were purified and stimulated in vitro 1, 2, and 4 wk after immunization with 100 μg/ml of KLH for 4 days, and proliferative responses were measured. The results are shown as the means of three mice ± SD. Ratios of proliferation (cpm) of splenic T cells from wild-type to that from FcRγ−/− mice were calculated at each sensitization time point, and the numbers are indicated over each bar in parentheses. B, Effect of Ag dose for immunization. Ten days after immunization with indicated doses of KLH, LN CD4+ T cells (3 × 10^5/well) were purified and stimulated in vitro with 1 μg/ml of KLH for 4 days, and proliferative responses were measured. The results are shown as the mean of three mice ± SD. Ratios of the proliferation of T cells from wild-type to that from FcRγ−/− mice were calculated at each immunization dose similar to A, and the numbers are indicated over each bar in parentheses. C, Cytokine production by T cells from FcRγ−/− mice. CD4+ T cells were stimulated with 100 μg/ml of KLH for 72 h under a similar condition as described for Fig. 2A, and the culture supernatants were collected and analyzed for concentrations of IL-2 and IFN-γ by ELISA. Note that IL-4 was below a detectable level in both mice. No cytokine production was detected in the absence of KLH. Data are representative of three independent experiments. **, p < 0.01; *, p < 0.05; compared with wild-type mice.

Regardless of the duration of immunization, the proliferative responses by T cells from FcRγ−/− mice were significantly lower than those of wild-type mice. Although the proliferative response decreased with time after immunization, the ratio of the proliferation of CD4+ T cells from wild-type mice to that from FcRγ−/− mice increased.

We also analyzed the effect of Ag dose on the priming of CD4+ T cells. Wild-type and FcRγ−/− mice were immunized with various doses of KLH, and CD4+ LN T cells were assessed for proliferation 10 days later. As shown in Fig. 3B, CD4+ T cells from FcRγ−/− mice exhibited lower responses with all Ag doses used. Although the proliferation of CD4+ T cells decreased at lower doses of Ags, the ratio of the proliferation of CD4+ T cells from wild-type mice to that from FcRγ−/− mice increased.

Collectively, these results suggest that the efficiency of Ag presentation to CD4+ T cells for proliferation increases in the presence of FcRγ in vivo, particularly under the condition of low doses of Ag and longer periods after immunization.
Suppression of Th cell-mediated cytokine and Ab production in FcRγ−/− mice

Because DTH and proliferation were impaired in FcRγ−/− mice, we examined other Th cell functions such as cytokine production and Ag-specific Ab formation. Defects in IL-2 and IFN-γ production by CD4+ T cells from FcRγ−/− mice were also observed, which were well correlated with the impaired proliferative response, although IL-4 production was below detection in both mice (Fig. 3C).

We also measured the titers of anti-KLH Abs in sera after immunization of wild-type and FcRγ−/− mice with KLH. Sera were obtained 2 wk after immunization, and the relative titers of anti-KLH Abs were determined by ELISA as a control, and the mean ± SD of 7–12 mice per group is shown. **, p < 0.01 compared with wild-type mice (A) or FcRγ−/− mice (B).

Normal primary T cell responses and Ag presentation in FcRγ−/− mice

The impaired T cell responses in FcRγ−/− mice could be attributed to a defect in either T cell function or Ag presentation. To clarify the basis for this, we first analyzed the T cell function in FcRγ−/− mice.

Because T cell functions in FcRγ−/− mice have not been analyzed previously except for intestinal intraepithelial T cells (8) and NK1.1+ T cells (9), both of which express FcRγ within the TCR complex, we examined the in vitro primary T cell responses upon stimulation by crosslinking with anti-TCRβ mAb and anti-CD28 mAb, superantigen (SAg), and MLR. As shown in Fig. 5, proliferative responses of CD4+ T cells from FcRγ−/− mice upon stimulation with immobilized anti-TCRβ mAb and anti-CD28 mAb (Fig. 5B) were almost the same as those of CD4+ T cells from wild-type mice. The low response to SAg in vivo was probably due to the C57BL/6 background of these mice. Furthermore, MLR by CD4+ T cells from FcRγ−/− mice did not differ from those of normal mice (Fig. 5C). In addition, we investigated the in vivo responses to SAg to address the question of whether the in vivo primary T cell response to SAg in FcRγ−/− mice is impaired, to compare with the impaired secondary response to nominal Ag. It has been shown that in vivo administration of SEB induces strong proliferation of Vβ8+ T cells and then a decrease of these cells due to apoptosis (19). We followed this protocol by administering SEB i.v. and analyzing the proportion of Vβ8+ T cells in spleen 2 days later as shown in Fig. 5D, the induction of proliferation
at the initial phase was almost the same for T cells from both FcRγ−/− and wild-type mice.

These results indicate that primary T cell functions in FcRγ−/− mice are normal and therefore suggest that impaired T cell responses of DTH, proliferation, and cytokine production upon immunization with Ag are not due to any T cell defect but rather can be attributed to insufficient priming upon secondary stimulation.

Because T cell function from FcRγ−/− mice was not impaired, we then investigated the function of Ag presentation to T cells by APCs from FcRγ−/− mice in vitro. OVA-specific murine T cell hybridoma DO11.10 was cultured with irradiated splenocytes from wild-type and FcRγ−/− mice in the presence of OVA peptide 323–339 (Fig. 6A) or whole OVA (Fig. 6B), and IL-2 secretion was assessed. The IL-2 production from the hybridoma cocultured with APCs from FcRγ−/− mice was almost equivalent to that from wild-type mice irrespective of the requirement of Ag processing. These results reveal that there is no difference in APC function between FcRγ−/− and wild-type mice.

Contribution of ICs for reduced T cell response in FcRγ−/− mice

It has been previously reported that IC composed of Ag and specific IgG Ab was efficiently taken up via FcR-mediated endocytosis by APCs such as DCs and macrophages, resulting in efficient Ag presentation to CD4+ T cells in vitro (20–22). Therefore, the reduction of T cell responses due to impaired priming in FcRγ−/− mice could be attributed to the failure of IC-mediated triggering of FcR or to a defect of unknown IC-independent function of FcR in Ag presentation.

To investigate whether in vivo low responses of CD4+ T cells from FcRγ−/− mice were due to a defect of FcR-mediated endocytosis of IC, we analyzed the Ag-specific response of CD4+ T cells from μMT mice in which B cell development has been shown to be severely impaired and the level of Abs was extremely low (15). μMT mice were immunized with KLH, and the proliferative response of CD4+ splenic T cells was analyzed in vitro upon stimulation with KLH in the presence of wild-type APCs. As shown in Fig. 7A, the proliferative response of CD4+ T cells from μMT mice was also impaired as compared with that from wild-type mice, and it was almost equivalent to that of FcRγ−/− mice. Furthermore, we observed that μMT mice exhibited reduced DTH response (data not shown). On the other hand, there was no significant difference in proliferative response by stimulation with PMA/A23187 among T cells from FcRγ−/−, wild-type, and μMT mice (Fig. 7B). These results suggest that IC and FcR play an important role in the effective Ag presentation to CD4+ T cells in an Ab-dependent manner in vivo.

Discussion

Although recent works have demonstrated that the triggering of inflammatory type I, II, and III responses was greatly dependent on FcRs (3–7), the role of FcR in the inflammatory type IV response still remains unclear. In this study, we determined the role of FcRs in DTH response using FcRγ−/− mice and demonstrated that the in vivo DTH response was clearly impaired in FcRγ−/− mice. This impaired response was seen not only in DTH but also in many
other Th cell functions such as T cell proliferation, cytokine production, and Ab production as secondary responses. These impaired T cell responses could be attributed to a defect either in T cells or in the Ag presentation to T cells, and our analysis points to the latter.

We first analyzed the function of FcRγ in T cells, which has not yet been analyzed extensively. The FcRγ transcript is not detectable in the normal T cell population even by RT-PCR (our unpublished observation). In this study, we showed that T cell responses in FcRγ−/− mice upon stimulation by TCR crosslinking, MLR, and SAg were not different from those of normal T cells. In addition, the in vitro proliferative responses of CD4+ T cells in FcRγ−/− mice (WT, and µMT mice were stimulated in vitro with PMA (50 ng/ml) and A23187 (100 ng/ml) for 2 days, and proliferative responses were measured. The results are shown as the mean of four mice ± SD. Data represent three independent experiments. **, p < 0.01; *, p < 0.05, compared with wild-type mice.

FIGURE 7. Suppression of proliferation of CD4+ T cells in µMT mice upon Ag stimulation. A, in vitro secondary proliferative responses of CD4+ T cells to KLH. Three weeks after immunization, splenic CD4+ T cells were purified from FcRγ−/− mice (●), µMT mice (■), and wild-type mice (○) stimulated in vitro (1 × 10⁶/well) with indicated concentrations of KLH for 4 days, and proliferative responses were measured. B, in vitro proliferative responses of CD4+ T cells to PMA/A23187. Three weeks postimmunization, purified CD4+ T cells (5 × 10⁶/well) from FcRγ−/− mice, wild-type mice (WT), and µMT mice were stimulated in vitro with PMA (50 ng/ml) and A23187 (100 ng/ml) for 2 days, and proliferative responses were measured. The results are shown as the mean of four mice ± SD. Data represent three independent experiments. **, p < 0.01; *, p < 0.05, compared with wild-type mice.

An alternative possibility for the observed impairment in DTH and proliferation of CD4+ T cells in FcRγ−/− mice may be a defect in APC function, because APCs such as macrophages and DCs express FcγRI and FcγRII (3, 4, 13, 23). It has been previously reported that APCs including DCs and macrophages are able to present Ags with 100-fold higher efficiency in the presence of Ag-Ab IC. This is because IC was taken up more efficiently via FcR-mediated endocytosis by APCs than by Ag alone, resulting in an efficient Ag presentation to CD4+ T cells in vitro (20–22, 24, 25). A defect in FcR-dependent enhancement of Ag presentation to CD4+ T cells may lead to a low response of T cells in FcRγ−/− mice in vivo. The enhancement of Ab responses has recently been demonstrated by the in vivo administration of in vitro preformed Ag-Ab ICs (26). However, because such a system with artificially preformed ICs does not reflect the physiological condition of the Ab response after immunization with Ag, the function of naturally formed IC under physiological condition has not been analyzed. In our study, we demonstrated for the first time that FcRγ−/− and Ab-deficient mice exhibit suppression of Th cell responses and Ab production under the condition of a physiological immunization process.

Furthermore, our results revealed a decrease in the Ag-specific proliferative responses of CD4+ T cells in µMT mice. Previously, Epstein et al. (27) reported that T cells in µMT mice exhibited normal functions in allo-responses. Similar to T cell responses in FcRγ−/− mice, it is likely that primary T cell responses are normal, whereas secondary responses are suppressed in µMT mice. Because ICs cannot be formed in µMT mice, these results suggest that IC-mediated Ag presentation is required for effective priming of CD4+ T cells. This was further supported by the observation that APCs from FcRγ−/− and wild-type mice could equally stimulate in vitro T cell clones in which no specific Ab was present. However, we cannot exclude the possibility that APC function was reduced in the absence of B cells in vivo, which µMT mice lack completely, although B cells are less effective APCs than DCs or macrophages.

The present analysis demonstrated for the first time that FcR plays an important role in augmenting the Ag-presenting capacity of DC or macrophages through an IC-dependent mechanism in vivo, even though this has already been assumed to be the case from in vitro studies. Preliminary results showing that even when macrophages or DCs were isolated from FcRγ−/− mice, these cells did not exhibit any difference in T cell proliferation, suggested that Ag presentation is augmented only in the presence of ICs. Our data showing that T cells from FcRγ−/− mice immunized 1 wk before readily exhibited a decrease in the response suggest that functional ICs could be formed during such an early period. In addition, we showed that the ratio of the proliferative response from wild-type mice to that from FcRγ−/− mice increased with time and lower Ag dose. These results suggest that the interaction of a low level of IC with FcR may affect the generation of memory responses in vivo. Our observation that FcR augments secondary T cell responses but not primary responses is also supported by the strong suppression of IgG response but not IgM production in FcRγ−/− mice. Because the helper function of CD4+ T cells is required for Ag-specific IgG but not IgM production, the failure of efficient priming of CD4+ T cells is responsible for the low level of Ag-specific IgG in FcRγ−/− mice.

Considering that FcγRI, FcγRII, and FcγRII are not expressed on the cell surface of various cells in FcRγ−/− mice (3, 4), it is likely that FcγRI and/or FcγRIII are responsible for effective Ag presentation in vivo. This is consistent with the observations that human FcγRI enhanced Ag presentation to T cells in vitro (28, 29) and that Ab production was augmented in transgenic mice expressing human FcγRI (30). It has been shown that crosslinking of FcγRIIB together with B cell receptor (FcεRI) inhibits the activation of B cells (mast cells) (31–33). Whether IC also inhibits the efficiency of Ag presentation through FcγRIIB is not yet known and has to be studied. Because it still remains unclear which FcR contributes to efficient presentation, further analysis using knockout mice for FcγRI, FcγRII, and FcγRIII on Ag presentation in
our system might uncover a pivotal function of FcR in the immune system.

As immunization progresses and specific Abs are produced, both augmentation through FcγRI/III and inhibition through FcγRIIB may occur. Thus, the total regulatory balance between such augmentation and suppression remains to be investigated to reach a better understanding of the physiological dynamics of immune responses.

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