

Critical Role of the Fc Receptor γ -Chain on APCs in the Development of Allergen-Induced Airway Hyperresponsiveness and Inflammation¹

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The FcR common γ -chain (FcR γ) is an essential component of the receptors Fc ϵ RI, Fc γ RI, and Fc γ RIII, which are expressed on many inflammatory cell types. The role of these receptors in the initiation or maintenance of allergic inflammation has not been well defined. FcR γ -deficient (FcR $\gamma^{-/-}$) and control (wild-type (WT)) mice were sensitized and subsequently challenged with OVA. Following sensitization and challenge to OVA, FcR γ -deficient (FcR $\gamma^{-/-}$) mice developed comparable levels of IgE and IgG1 as WT mice. However, numbers of eosinophils, levels of IL-5, IL-13, and eotaxin in bronchoalveolar lavage fluid, and mononuclear cell (MNC) proliferative responses to OVA were significantly reduced, as was airway hyperresponsiveness (AHR) to inhaled methacholine. Reconstitution of FcR $\gamma^{-/-}$ mice with whole spleen MNC from WT mice before sensitization restored development of AHR and the numbers of eosinophils in bronchoalveolar lavage fluid; reconstitution after sensitization but before OVA challenge only partially restored these responses. These responses were also restored when FcR $\gamma^{-/-}$ mice received T cell-depleted MNC, T and B cell-depleted MNC, or bone marrow-derived dendritic cells before sensitization from FcR $^{+/+}$ or Fc γ RIII-deficient but not FcR $\gamma^{-/-}$ mice. The expression levels of Fc γ RIV on bone marrow-derived dendritic cells from FcR $^{+/+}$ mice were found to be low. These results demonstrate that expression of FcR γ , most likely Fc γ RI, on APCs is important during the sensitization phase for the development of allergic airway inflammation and AHR. *The Journal of Immunology*, 2007, 178: 480–488.

Various inflammatory cell types, including lymphocytes, eosinophils, neutrophils, mast cells, macrophages, and dendritic cells (DC)⁵ are involved in the development of allergic airway inflammation and airway hyperresponsiveness (AHR), major elements of bronchial asthma. Most of these immune/inflammatory cells, except for T lymphocytes, express receptors for the Fc chain of Ig (FcR) and activation of these cells is in large part the result of signaling through these receptors, which are members of the multichain immune recognition receptor family. As a result, the FcRs may play a pivotal role in the develop-

ment of allergic airway disease. Among the FcRs, there is a balance of activities with receptors exhibiting either positive or negative regulation of immune cell responses. The FcR common γ -chain (FcR γ) is a key component of several of these receptors. The high-affinity IgE receptor (Fc ϵ RI), the high-affinity IgG receptor (Fc γ RI), and the low-affinity IgG receptors (Fc γ RII, Fc γ RIII, and recently identified Fc γ RIV) all contain an ITAM that is essential for immune cell activation through cytosolic protein kinases (1–3). Many of these cell types also express a unique inhibitory FcR for IgG, Fc γ RIIb, which contains an ITIM in its cytoplasmic domain. Engagement of the activating FcRs triggers many biological functions including phagocytosis, degranulation, cytolysis, and the transcriptional activation of genes encoding many of the cytokines involved in allergic lung inflammatory responses. In addition, FcRs mediate the uptake of immune complexes, their degradation, and, ultimately, peptide Ag presentation on macrophages and DC (4, 5).

In bronchial asthma, the role of allergen-specific Abs is important, and serum levels of allergen-specific IgE and disease severity have been closely correlated. As a corollary, anti-IgE therapy in severe atopic asthmatics has been demonstrated to be effective (6). However, in a number of studies in mice, a role for allergen-specific Abs has not been demonstrated nor required for the development of AHR and allergic airway inflammation (7–10). In addition to the high-affinity receptor for IgE, expression of Fc ϵ RII (CD23), the low-affinity IgE receptor may also be involved in the development of airway eosinophilia (11). Indirectly, the efficacy of i.v. Ig treatment in severe asthma (12) may indicate that inhibitory FcR-mediated activation of cells by IgG or immune complexes can be a potent regulator of allergic airway inflammation and that targeting the FcR pathway could control airway allergic responses.

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⁵ Abbreviations used in this paper: DC, dendritic cell; AHR, airway hyperresponsiveness; WT, wild type; BAL, bronchoalveolar lavage; PBLN, peribronchial lymph node; MNC, mononuclear cell; PAS, periodic acid-Schiff; MBP, major basic protein; MCh, methacholine; RL, lung resistance; BM-DC, bone marrow-derived DC.

To determine the role of activating-type FcRs in the development of allergen-induced AHR and airway inflammation, we investigated the responses of Fc γ -deficient (Fc γ ^{-/-}) mice following allergen sensitization and challenge. Fc γ ^{-/-} mice failed to develop AHR or allergic airway inflammation, and this deficiency was localized to abnormalities of APC function.

Materials and Methods

Animals

Fc γ -deficient (Fc γ ^{-/-}) mice from 6 to 8 wk of age were used. These mice were derived as described previously (13). As controls, Fc γ -sufficient C57BL/6 (wild-type (WT)) mice and Fc γ RIII-deficient (Fc γ RIII^{-/-}) mice were purchased from The Jackson Laboratory or Charles River Laboratories. Mice with a disruption of the *JH* gene (B cell^{-/-}, C57BL/6 background) (14) were provided by Dr. L. Wysocki (National Jewish Medical and Research Center, Denver, CO). All mice were maintained on an OVA-free diet under specific pathogen-free conditions. Experiments were conducted under a protocol approved by the Institutional Animal Care and Use Committee of Okayama University Medical School and the National Jewish Medical and Research Center.

Sensitization and airway challenge

Mice received the following treatments: PBS/OVA, challenged alone to aerosolized OVA; OVA/OVA, sensitized to OVA (together with alum) followed by aerosolized airway challenge with nebulized OVA. Sensitization was achieved by immunization i.p. with 20 μ g of OVA (grade V; Sigma-Aldrich) emulsified in 2.25 mg of alum (Imject Alum; Pierce) in a total volume of 100 μ l on days 1 and 14. Mice were challenged via the airways with OVA (1% in saline) for 20 min on days 28, 29, and 30 by ultrasonic nebulization (NE-U07; OMRON) and assessed 48 h after the last OVA exposure.

Measurement of serum levels of anti-OVA Ab

Peripheral blood samples were collected by cutting the inferior vena cava after measurements of airway responsiveness, and serum was separated. Serum levels of OVA-specific-IgE and IgG1 were determined by ELISA (15). The Ab titers of the samples were related to pooled standards containing OVA-specific IgE and IgG1 generated from OVA-sensitized and -challenged BALB/c mice and expressed as ELISA units per milliliter.

Bronchoalveolar lavage (BAL) and measurement of cytokine levels

BAL fluid was collected with 1 ml of saline via the tracheal tube 48 h after the last airway challenge. For differential cell counts, slides were prepared with a Cytospin III (Thermo Shandon) and cells were stained with May-Giemsa. BAL fluid cytokine levels for IL-5, IL-4, IL-13, and eotaxin levels were determined by ELISA (R&D Systems).

Spleen and peribronchial lymph node (PBLN) mononuclear cell (MNC) proliferation

MNC from spleen and PBLN were purified by density gradient centrifugation (Histopaque-1083; Sigma-Aldrich). Cells were counted and resuspended in culture medium (IMDM; Invitrogen Life Technologies) containing heat-inactivated FCS (Sigma-Aldrich), 2-ME (Sigma-Aldrich), 100 U/ml penicillin (Banyu Pharmaceutical), and 100 μ g/ml streptomycin (Meiji Seika Kaisha). Cells were plated at 1×10^6 /ml in 96-well round-bottom tissue culture plates in triplicate and incubated with medium alone or containing OVA (1, 10, and 100 μ g/ml) for 72 h. Cell proliferation was assessed by uptake of [³H]thymidine, which was added to cell culture wells for the last 6 h of the incubation period. After incubation, cells were harvested onto a glass-fiber filter paper using a cell harvester apparatus and the incorporated radiolabel was counted using a gamma spectrometer (TRI-CARB 2260XA; Packard Japan).

Histological studies

Forty-eight hours after the last airway challenge, lungs were inflated with 1 ml of 10% formalin through the trachea, and fixed in formalin by immersion. Tissue blocks of lung tissue from four to five mice in each group were cut from around the main bronchus and embedded in paraffin blocks; two to three tissue sections (5 μ m) per mouse were then affixed to microscope slides and deparaffinized. The slides were then stained with H&E or periodic acid-Schiff (PAS). Cells containing eosinophilic major basic protein (MBP) were identified by immunohisto-

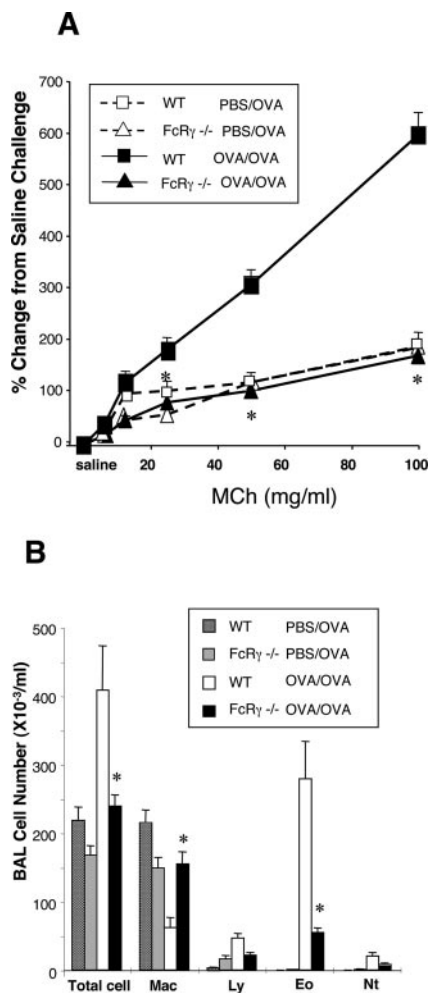


FIGURE 1. Response of Fc γ ^{-/-} mice to sensitization and challenge. **A**, RL in response to inhaled MCh. **B**, Cell composition of BAL fluid. WT mice or Fc γ ^{-/-} mice were sensitized and challenged with OVA (OVA/OVA) or challenged only (PBS/OVA). Mac, Macrophage; Ly, lymphocyte; Eo, eosinophil; Nt, neutrophil. The results for each group are expressed as mean $\pm$ SEM ($n = 8$). *, Significant differences ($p < 0.05$) Fc γ ^{-/-} mice OVA/OVA vs WT mice PBS/OVA.

chemical staining as previously described using rabbit anti-mouse MBP (provided by Dr. J. J. Lee, Mayo Clinic, Scottsdale, AZ) (15). Photographs of the slides were taken with a microscope (BX40; Olympus) equipped with a digital camera (Q-color 3; Olympus) and images were stored on a Macintosh computer. The numbers of eosinophils were counted and adjusted by area. Goblet cell metaplasia was quantified as the number of pixels on the computer converted from PAS⁺ areas along the airway epithelium. The quantification was performed using NIH Scion Image software (version 1.63), available on the Internet at (<http://rsb.info.nih.gov/nih-image>). Six to eight different fields per slide were examined in a blinded manner.

Determination of airway responsiveness

Airway responsiveness was assessed as changes in airway function after challenge with aerosolized methacholine (MCh; Sigma-Aldrich). Mice were anesthetized, tracheostomized, mechanically ventilated, and lung function was assessed as described previously (16). Ventilation was achieved at 160 breaths per minute at a tidal volume of 0.16 ml with a positive end-expiratory pressure of 2–4 cm H₂O with a ventilator (SN-480-7; Shinano Seisakusho). Lung resistance (RL) was continuously computed (Labview; National Instruments) by fitting flow, volume, and pressure to an equation of motion using a recessive least-squares algorithm.

Aerosolized MCh was administered through bypass tubing via an ultrasonic nebulizer (model 5500D; DeVilbiss) placed between the expiratory port of the ventilator and the four-way connector. Aerosolized MCh was

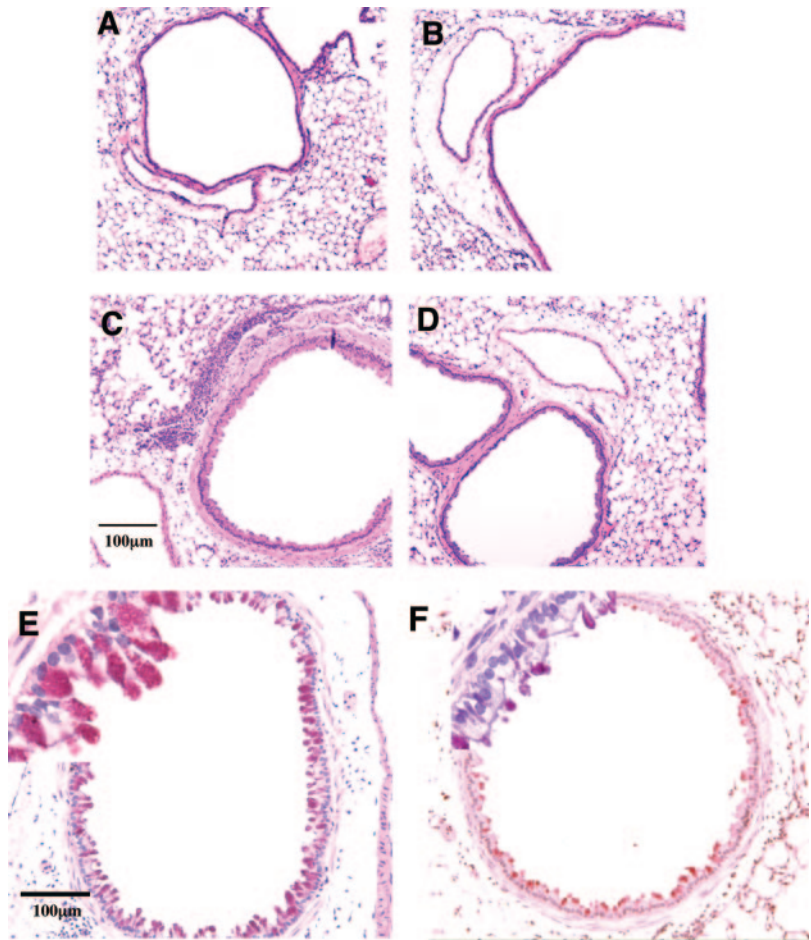


FIGURE 2. Lung histology. Histological examination of lung tissue from WT and FcR $\gamma^{-/-}$ -challenged-only (PBS/OVA), sensitized, and challenged with OVA (OVA/OVA) mice. Lung sections were stained with H&E (A–D) or PAS (E and F). A, WT (PBS/OVA); B, FcR $\gamma^{-/-}$ (PBS/OVA); C, WT (OVA/OVA); D, FcR $\gamma^{-/-}$ (OVA/OVA); E, WT (OVA/OVA); and F, FcR $\gamma^{-/-}$ (OVA/OVA). Original magnification, $\times 200$.

administered for 8 s with a tidal volume of 0.45 ml and frequency of 60 breaths per minute using another ventilator (model 683; Harvard Apparatus). The data of RL were continuously collected for up to 3 min and maximum values were taken.

Adoptive transfer of spleen cells

Spleen MNC were purified by gradient centrifugation, resuspended in PBS, and transferred to recipient mice through the tail vein (1×10^7 cells in 200 μ l). Five hours after cell transfer, mice were sensitized to OVA or challenged with OVA in previously sensitized mice. In some MNC preparations, T cells were depleted (T cell-depleted) from either WT or B cell-

deficient (T and B cell-depleted) mice by treatment with anti-Thy1.2 Ab (H.0.13) and rabbit complement (Low-Tox-M; Cedarlane Laboratories). Following treatment, the number of CD3 $^{+}$ cells was $<5\%$ in both WT and B cell $^{-/-}$ mice, respectively; B220 $^{+}$ cells were 88 and 3% in WT and B cell $^{-/-}$, respectively; NK cells were 3 and 50%, respectively; and CD11c $^{+}$ were 4 and 20%, respectively.

Adoptive transfer of bone marrow-derived DC (BM-DC)

Bone marrow-derived DC (BM-DC) from WT, FcR $\gamma^{-/-}$, or Fc γ RIII $^{-/-}$ mice were generated as described previously (17), with some modifications. Briefly, bone marrow cells obtained from femurs and tibias of mice

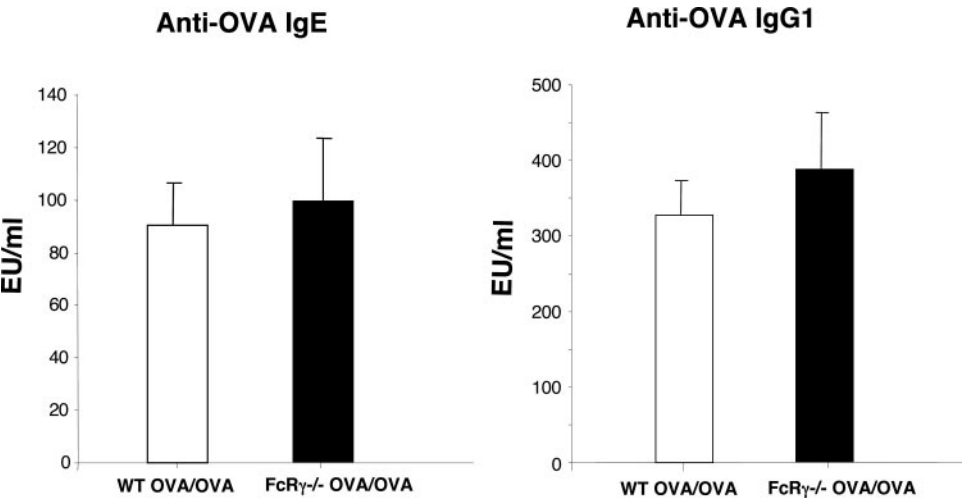
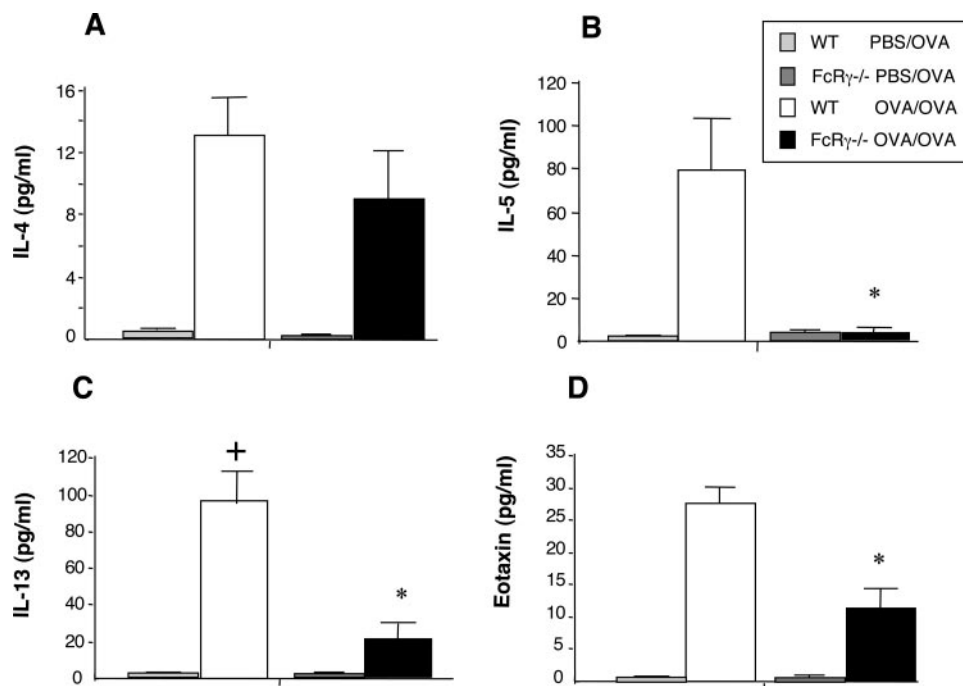


FIGURE 3. OVA-specific Ab levels in serum. The results for each group are expressed as mean $\pm$ SEM ($n = 8$). EU/ml, ELISA units per milliliter. OV/OVA, sensitized and challenged to OVA.

FIGURE 4. Cytokine levels in BAL fluid. *A*, IL-4; *B*, IL-5; *C*, IL-13; and *D*, eotaxin. The results for each group are expressed as mean $\pm$ SEM ($n = 8$). PBS/OVA, challenged only to OVA. OVA/OVA, sensitized and challenged to OVA. *, Significant differences ($p < 0.05$) between $\text{Fc}\gamma\text{R}^{-/-}$ OVA/OVA mice vs WT OVA/OVA mice. +, Significant differences ($p < 0.05$) between WT OVA/OVA to WT or $\text{Fc}\gamma\text{R}^{-/-}$ PBS/OVA.



were placed in culture flasks at 37°C in DC culture medium (RPMI 1640 containing 10% heat-inactivated FCS, 50 μM 2-ME, 2 mM L-glutamine, 100 U/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin (Invitrogen Life Technologies), 10 ng/ml recombinant mouse GM-CSF, and 10 ng/ml recombinant mouse IL-4 (R&D Systems)). After 2 h of culture, nonadherent cells were collected and continued in culture. On day 5, nonadherent cells were collected again and resuspended in fresh DC culture medium. On day 7, the nonadherent cells were collected and 0.5×10^6 were injected i.p. into recipient mice before sensitization. More than 90% of the transferred cells expressed CD11c. To determine the expression levels of $\text{Fc}\gamma\text{RIV}$ on BM-DC, cells were stained with anti- $\text{Fc}\gamma\text{RIV}$ mAb (9E9-Alexa Fluor 647, provided by Drs. J. Ravetch and F. Nimmerjahn, The Rockefeller University, New York, NY) and analyzed by flow cytometry (FACSCalibur; BD Biosciences).

Statistical analysis

All results were expressed as the mean $\pm$ SEM as a standard method of presentation for this type of data. The t test was used to determine differences between two groups and the Tukey-Kramer test was used for com-

parisons between multiple groups. Measured values may not be normally distributed and due to the small sample sizes this is difficult to test for. Nonparametric analysis using the Mann-Whitney U test was also used to confirm that the statistical differences remained significant even if the underlying distribution was uncertain. The p values for significance were set to 0.05 for all tests.

Results

Airway responsiveness and allergic airway inflammation in the $\text{Fc}\gamma\text{R}$ -deficient mice

As shown in Fig. 1, following sensitization and challenge with OVA, WT mice developed increases in lung resistance to inhaled MCh in a dose-dependent manner and a marked increase in BAL total cell numbers, comprised in large part by increased numbers of eosinophils when compared with challenged-only mice. $\text{Fc}\gamma\text{R}^{-/-}$ mice failed to show such increases in lung resistance following sensitization and challenge and demonstrated significantly lower

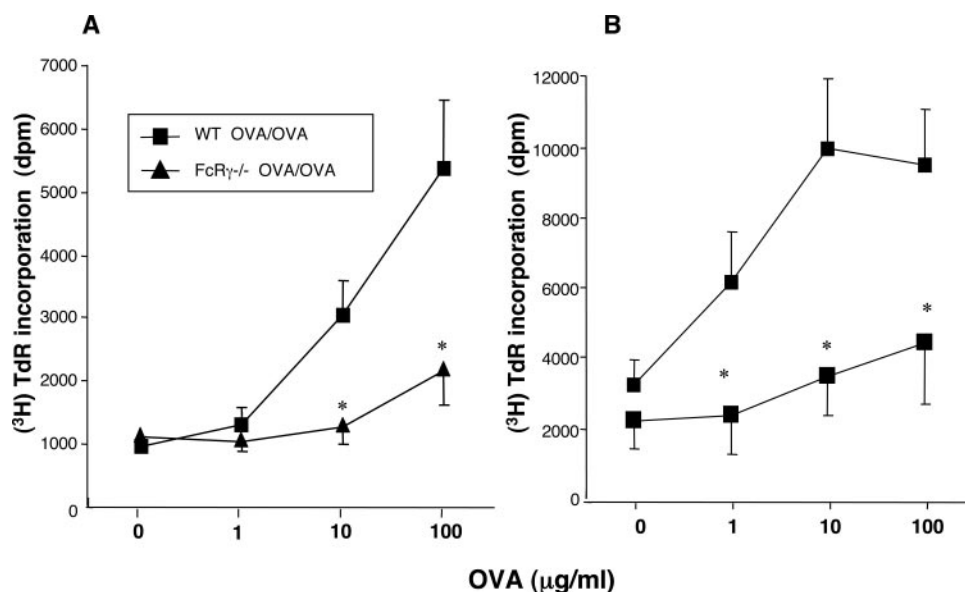
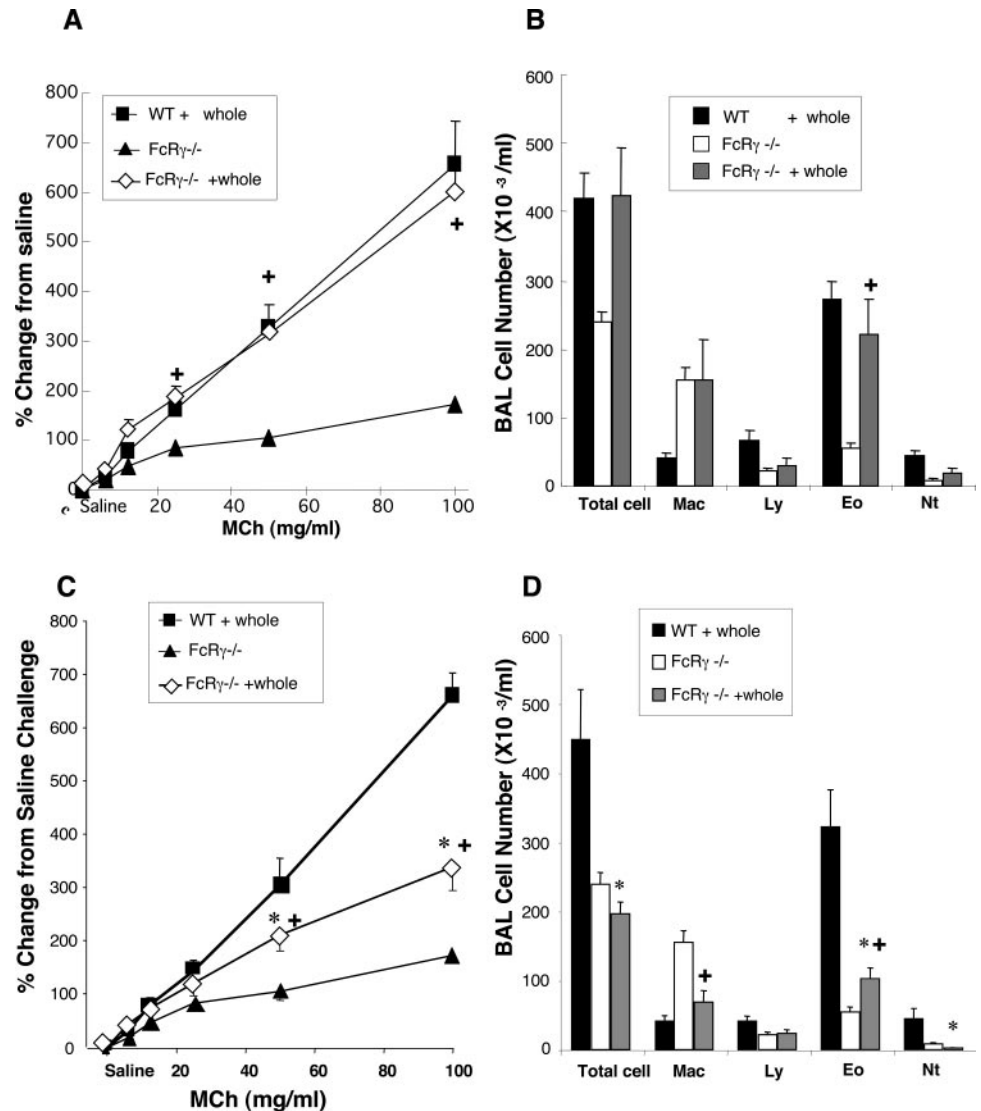


FIGURE 5. MNC proliferation. *A*, Spleen MNC or *B*, PBLN MNC. Proliferative responses to OVA were evaluated by uptake of tritiated thymidine and shown as dpm. Data are expressed as mean $\pm$ SEM ($n = 8$). OVA/OVA, sensitized and challenged to OVA. *, Significant differences ($p < 0.05$) between $\text{Fc}\gamma\text{R}^{-/-}$ OVA/OVA mice vs WT OVA/OVA mice.

FIGURE 6. Effect of adoptive transfer of spleen MNC. Spleen MNC from WT mice were transferred to mice before sensitization or after sensitization and before challenge. **A** and **B**, FcR $\gamma^{-/-}$ and WT received whole-spleen MNC before sensitization followed by challenge to OVA (FcR $\gamma^{-/-}$ + whole and WT + whole) and compared with OVA-sensitized and -challenged FcR $\gamma^{-/-}$ mice (FcR $\gamma^{-/-}$). **C** and **D**, FcR $\gamma^{-/-}$ and WT received whole-spleen MNC after sensitization but before challenge to OVA (FcR $\gamma^{-/-}$ + whole and WT + whole) and compared with OVA-sensitized and -challenged FcR $\gamma^{-/-}$ mice (FcR $\gamma^{-/-}$). **A** and **C**, RL in response to inhaled MCh. **B** and **D**, Cellular composition in BAL fluid. Mac, Macrophage; Ly, lymphocyte; Eo, eosinophil; Nt, neutrophils. The results for each group are expressed as mean $\pm$ SEM ($n = 8$). *, Significant differences ($p < 0.05$) between FcR $\gamma^{-/-}$ + whole-spleen mice vs WT + whole-spleen mice. +, Significant differences ($p < 0.05$) between FcR $\gamma^{-/-}$ mice + whole-spleen vs FcR $\gamma^{-/-}$ mice.



numbers of eosinophils in the BAL fluid (and total cell counts) when compared to WT mice.

Histological assessment of lung tissue

In parallel to the findings in BAL fluid, H&E-stained lung tissue sections showed increased numbers of inflammatory cells, including eosinophils, in the peribronchial regions in sensitized and challenged WT mice compared with challenged-only WT mice (Fig. 2, *A* and *C*). In contrast, sensitization and challenge to OVA did not induce a similar accumulation of inflammatory cells in the lung tissue of FcR $\gamma^{-/-}$ mice (Fig. 2, *B* and *D*). PAS-stained lung sections also revealed that FcR $\gamma^{-/-}$ mice failed to develop the degree of airway goblet cell metaplasia observed in WT mice after allergen sensitization and challenge (Fig. 2, *E* and *F*).

Humoral responses to sensitization and challenge with OVA

As shown in Fig. 3, serum levels of anti-OVA IgE and IgG1 in FcR $\gamma^{-/-}$ mice were comparable to WT mice after sensitization and challenge with OVA. These Abs were not detectable in the challenged-only WT or FcR $\gamma^{-/-}$ mice (data not shown). The Ab data confirmed the ability of these deficient mice to respond to sensitization and challenge, at least in some of the responses.

Cytokine levels in the BAL fluid

After sensitization and challenge to OVA, IL-4, IL-5, IL-13, and eotaxin levels in the BAL fluid were measured (Fig. 4). In the sensitized and challenged WT mice, levels of all four proteins increased compared with challenged-only mice. IL-4 levels in BAL fluid were barely detectable and no significant differences were observed in the levels of IL-4 in BAL fluid of sensitized and challenged FcR $\gamma^{-/-}$ and WT mice. The levels of IL-5, IL-13, and eotaxin in sensitized and challenged FcR $\gamma^{-/-}$ mice were significantly lower. Levels of IFN- γ were similar in WT and deficient mice (data not shown).

MNC proliferation

As measured by tritiated thymidine incorporation, proliferative responses to OVA in MNC from the PBLN and spleen were significantly lower in FcR $\gamma^{-/-}$ mice compared to WT mice following sensitization and challenge (Fig. 5). PBLN cells from challenged-only mice were not available because the lymph nodes were small and cell yields very low.

Reconstitution of FcR $\gamma^{-/-}$ mice with spleen MNC

MNC were prepared from spleens of naive mice and adoptively transferred into FcR $\gamma^{+/+}$ or FcR $\gamma^{-/-}$ mice before sensitization

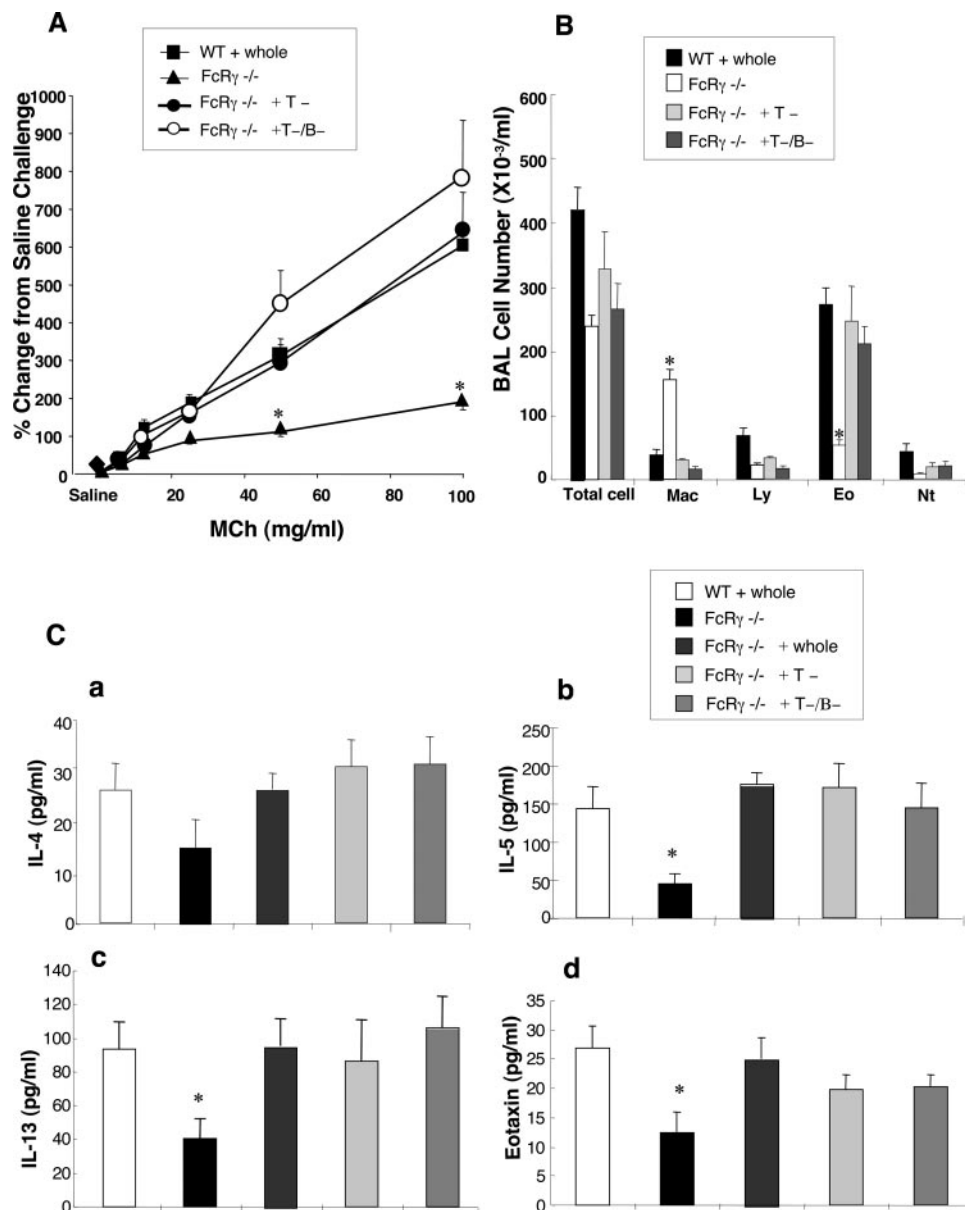


FIGURE 7. Effect of transfer of T or T and B cell-depleted spleen MNC. *A*, RL in response to inhaled MCh; *B*, Cellular composition in BAL fluid. *C*, Cytokine levels in BAL fluid. Spleen MNC transferred into WT mice (WT + whole). FcRγ^{-/-} mice receiving no cells (FcRγ^{-/-}), FcRγ^{-/-} mice that received T cell-depleted spleen MNC (FcRγ^{-/-} + T-), and FcRγ^{-/-} mice that received T cell-depleted spleen MNC from B cell-deficient mice (FcRγ^{-/-} + T-/B-). Mac, Macrophage; Ly, lymphocyte; Eo, eosinophil; Nt, neutrophil. The results for each group are expressed as mean ± SEM (*n* = 8). *, Significant differences (*p* < 0.05) between FcRγ^{-/-} mice receiving cells or WT mice vs FcRγ^{-/-} mice receiving no cells.

and challenge. Transfer of spleen MNC from FcRγ^{+/+} mice followed by sensitization and challenge to OVA restored both airway responsiveness and allergic airway inflammation in FcRγ^{-/-} mice (Fig. 6, *A* and *B*). When FcRγ^{-/-} mice received spleen MNC after sensitization but before OVA challenge, the extent of reconstitution of airway responsiveness, inflammation, and the number of eosinophils in BAL was significant (compared with mice challenged alone), but limited when compared with the results following their transfer before sensitization (Fig. 6, *C* and *D*). Transfer of spleen MNC from FcRγ^{-/-} mice into either WT or deficient mice failed to alter the responses (data not shown).

Effects of reconstitution with T or T and B cell-depleted spleen FcRγ^{+/+} MNC and FcγR-deficient BM-DCs

To determine which donor cell types expressing FcRγ may have contributed to the reconstitution effects of spleen MNC on development of AHR and allergic airway inflammation, FcRγ^{-/-} recipient mice were studied following transfer of T cell-depleted or T and B cell-depleted spleen MNC from FcRγ^{+/+}

mice before sensitization (Fig. 7, *A–C*). Both T cell and T/B cell-depleted FcRγ^{+/+} MNC restored AHR; the number of eosinophils; and the levels of IL-4, IL-5, IL-13, and eotaxin in BAL fluid to the same levels as following adoptive transfer of whole-spleen MNC into WT recipients. IL-4 levels were increased but in each group were low. There were no significant differences in any of the cytokine/chemokine levels between WT recipients and FcRγ^{-/-} recipients.

Based on the data using T/B-depleted spleen MNC preparations, it appeared that the deficiency in Fc receptors in FcRγ^{-/-} mice may be tied to defective APC function. To address this directly, BM-DC were differentiated from FcRγ^{+/+}, FcRγ^{-/-}, or FcγRIII^{-/-} mice and transferred into FcRγ^{-/-} mice. FcRγ^{-/-} recipients fully developed increases in RL (Fig. 8*A*), lung eosinophilia (Fig. 8, *B* and *C*), and goblet cell metaplasia (Fig. 8, *D* and *E*) when they received BM-DC from FcRγ^{+/+} and FcγRIII^{-/-} but not FcRγ^{-/-} mice. The expression levels of FcγRIV on BM-DC by flow cytometric analysis revealed very low levels of FcγRIV on BM-DC from WT mice compared with BM-DC from FcRγ^{-/-} (Fig. 8*F*).

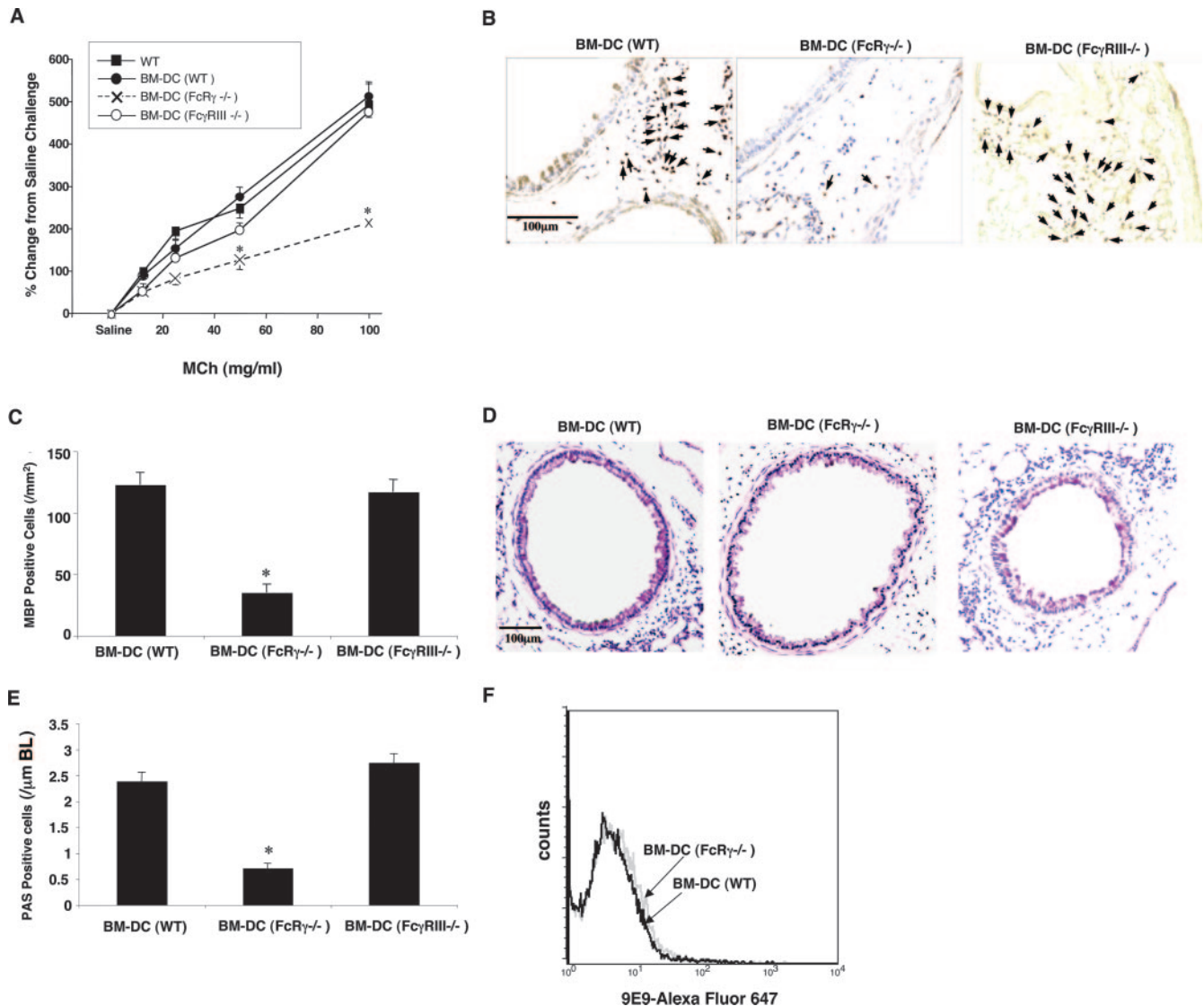


FIGURE 8. BM-DC reconstitute responses in FcR γ ^{-/-} mice. BM-DC were transferred into FcR γ ^{-/-} mice followed by sensitization and challenge to OVA. **A**, RL in response to inhaled MCh. **B**, MBP⁺ eosinophils in the submucosal area of large airways. **C**, Numbers of MBP⁺ eosinophils. **D**, Lung sections stained with PAS. **E**, Goblet cell quantitative analysis shown as PAS-positive areas in the lung tissue of FcR γ ^{-/-} mice that received BM-DC from FcR γ ^{-/-} mice (BM-DC (FcR γ ^{-/-})); FcR γ ^{-/-} mice that received BM-DC from Fc γ RIII^{-/-} mice (BM-DC (WT)); and FcR γ ^{-/-} mice that received BM-DC from WT mice (BM-DC (Fc γ RIII^{-/-})). **F**, The expression level of Fc γ RIV with anti-mouse Fc γ RIV Ab (9E9-Alexa Fluor 647) on BM-DC from WT mice and BM-DC from FcR γ ^{-/-} mice. BL, Basal lamina of the airway epithelium. The results for each group are expressed as mean $\pm$ SEM ($n = 8$). *, Significant differences ($p < 0.05$) compared with BM-DC (FcR γ ^{-/-}).

Discussion

Because of their widespread distribution on many of the cell types involved in allergic inflammatory responses in the lung, the prediction was that genetic manipulation of the activating-type FcRs would affect the consequences of allergen sensitization and challenge. This was demonstrated in the present study where FcR γ ^{-/-} mice were shown to develop significantly lower AHR to inhaled MCh as well as decreased BAL and lung tissue inflammation, eosinophilia, and goblet cell metaplasia. These decreased responses were accompanied by lower levels in the BAL fluid of IL-5, IL-13, and eotaxin, as well as decreased Ag-specific T cell proliferative responses. The results of these studies indicate that deletion of FcR γ results in the failure to develop allergen-induced AHR, eosinophilic inflammation, Th2 cytokine responses, and goblet cell metaplasia, all features of the response in mice normally expressing FcR γ . Somewhat surprising, and unexplained at present, were the

findings that allergen-specific Ab responses remained intact. Thus, despite the absence of FcR γ expression on B cells, the deficient mice were nonetheless able to respond to allergen sensitization and challenge with specific Ab production. In other systems as well, specific Ab production has been demonstrated in mice that lack FcR γ (18), or where APC function was inhibited (19).

The involvement of FcRs and those associated with FcR γ in particular have not been extensively examined in models of allergen-induced AHR and airway inflammation. Deletion of the FcR γ -chain results in the deficiency of activating-type FcRs, including Fc γ RI, Fc γ RIII, and Fc ϵ RI, but does not affect the expression or inhibitory function of Fc γ RIIb. Deletion of FcR γ also results in the deficiency of other receptors such as paired Ig-like receptor A, but the phenotype of paired Ig-like receptor A deficiency resulting from deletion of FcR γ is not clear (20). To some extent, it appears that the consequence of a specific deficiency of an activating-type

Fc γ R in mice depends on the approach taken to elicit AHR and airway inflammation. For example, following allergen challenge of sensitized mice, neutrophils are the first inflammatory cells found in BAL fluid. In Fc γ R^{-/-} mice, the number of neutrophils and levels of neutrophil chemokines in BAL fluid were significantly reduced compared with Fc γ R^{+/+} animals; absence of Fc ϵ RI was without effect on these responses (21). Furthermore, investigation of this neutrophil response showed that it was Ab-dependent and mediated through Fc γ RIII (21). Interestingly, development of AHR and eosinophilic inflammation following sensitization and challenge was independent of this neutrophil response, indicating that the failure to express Fc γ RIII in the Fc γ R^{-/-} mice could not account for the absence of AHR, inflammation, and goblet cell metaplasia (22).

Following 10 days of airway exposure to allergen in the absence of systemic sensitization or adjuvant, or following passive sensitization with allergen-specific IgE (or IgG1) followed by limited airway challenge, the development of AHR and airway inflammation was dependent on the expression of Fc ϵ RI (23). This is contrasted by the approach used in the present study where sensitization to allergen was systemic, together with alum as an adjuvant, followed by allergen challenge. In this case, there are no requirements for B cells or Abs, including allergen-specific IgE, Fc ϵ RI, or mast cells (9, 16). Nonetheless, in Fc γ R^{-/-} mice, sensitization and challenge failed to elicit AHR, lung eosinophilia, or goblet cell metaplasia. The absence of eosinophilia in the BAL fluid or lung tissue was likely associated with the significantly lower levels of IL-5 and eotaxin (24), whereas the marked decrease in goblet cell metaplasia was likely the consequence of the failure to produce adequate amounts of IL-13 (25).

Genetic deletion of Fc γ R has resulted in the attenuation or decreased susceptibility to various autoimmune diseases such as collagen-induced arthritis (26), vasculitis (27), glomerulonephritis (28), or Alzheimer's disease (29). Deficiency of the Fc γ R also has been shown to impair the development of NK cell-mediated Ab-dependent cytotoxicity (13) and to impair the development of anaphylaxis (30–32); in the absence of Fc γ RIIb, anaphylaxis was enhanced (33). One of the critical functions of Fc γ Rs is their ability to enhance Ag presentation by the APCs in the lung the DC (2, 34). Most Fc γ Rs efficiently internalize Ag-Ab complexes and induce the processing of Ags into peptides that are presented by MHC class I and class II molecules *in vitro* (2). In humans, Fc γ RIIa and Fc γ RIIb were shown to regulate DC function toward either activation or inhibition, respectively (35). This implies that Fc γ Rs have a pivotal role in augmenting humoral and cellular immune responses at the level of Ag presentation and at the initiation of the response.

Based on the role Fc γ Rs play in DC function, we showed that provision of spleen MNC, including T-depleted and T/B cell-depleted MNC, fully restored all of the allergen-induced responses in the lung, including altered airway function, inflammation, goblet cell metaplasia, and Th2 cytokine and chemokine levels. Because DC alone reconstituted the responses, the data were suggestive of defective DC function in the Fc γ R^{-/-} mice. This was confirmed in experiments generating BM-DC from Fc γ R^{+/+} or Fc γ R^{-/-} mice. To further address the role of the Fc γ chain in DC activity, DC reconstitution experiments were conducted in Fc γ R^{-/-} mice. DC from either Fc γ R^{+/+} or Fc γ RIII^{-/-} mice fully reconstituted all of the responses. As well, because DC from Fc γ R^{+/+} mice were found to express very low levels of Fc γ RIV, it was unlikely that Fc γ RIV contributed to the responses. Although, Nimmerjahn et al. (3) showed Fc γ RIV expression on BM-DC, because DC were cultured with IL-4 in this study it is possible that Th2-type cytokines may have down-regulated Fc γ RIV expression (3).

Taken together, all of the data related to Fc γ R function, including the findings that deficiency of Fc γ RI resulted in impaired Ag presentation (36, 37), the absence of expression of Fc ϵ RI on mouse DCs, the low expression of Fc γ RIV, and the reconstitution experiments point to the essential role of Fc γ RI in DC function for the full development of lung allergic responses.

There are two distinguishable phases for development of these lung allergic responses, the initial sensitization phase and the second airway challenge phase. APC function is almost certainly required at both stages of the response. Lung DC function likely predominates in the airway allergen challenge phase, whereas a number of APCs may be activated during the sensitization phase, especially when adjuvant is given together with allergen. For maximum reconstitution of all of the lung allergic responses, MNC or DC had to be transferred to recipients during the period of sensitization; when given after sensitization was completed, but before airway challenge, the responses in Fc γ R^{-/-} recipients were lower, but nonetheless were present. It is not entirely clear why this is the case other than to suggest that at least some of the priming that does occur during the sensitization phase, especially when alum is coadministered, has systemic effects including effects in the lung, even in the absence of Fc γ R. These findings support the necessity of Fc γ R at both phases for full development of the responses, but because transfer before challenge was effective, at least some of the sensitization could be achieved in the absence of Fc γ R. This is supported by the findings of normal Ag-specific Ab levels in the serum which develop almost entirely during the sensitization phase. Conceivably, the incorporation of alum in the sensitization phase bypasses some of the requirements for Fc γ R in this phase, and the normal Ab development may be related to the ability of alum alone to trigger IL-4 production (38).

Regnault et al. (5) showed that BM-DC derived from Fc γ R^{-/-} mice failed to mature normally or promote efficient MHC class I-restricted presentation of peptides from exogenous IgG-complexed Ags. Interestingly, presentation of soluble Ag (at higher concentrations than IgG-complexed Ags) was also impaired in Fc γ R-deficient DC. Fc γ -dependent Ag uptake by DC in the absence of specific IgG Ab may also represent a form of cross-priming, which is evident in DC (5). In addition, Fc γ R ligation by non-Ig molecules such as C-reactive protein (39) or amyloid P (40), which increase during inflammatory responses and can opsonize particles, may also activate critical Fc γ -expressing cells. We previously described that B cell-deficient mice, which fail to make specific Ab of any isotype, fully develop AHR and airway inflammation following systemic allergen sensitization and airway challenge (9). It is not entirely clear what role Fc γ R plays in the absence of engagement of Fc γ R by IgG. However, following systemic sensitization and challenge or priming of DC with allergen before adoptive transfer and allergen challenge, Fc γ R has been shown to be essential to AHR development as suggested by inhibition of the Fc γ -related signaling molecule Syk (19). Furthermore, in some T cell subsets such as double-negative T regulatory cells, Fc γ R forming a complex with CD3 may play a functional role in these cells through activation of Syk (41). This may indicate a distinctive role for Fc γ R, independent of IgG ligation, as observed in B cell-deficient mice.

In summary, expression of Fc γ R is important in the development of allergen-induced AHR and inflammation. Beyond the role of Fc ϵ RI mediating mast cell activation, the importance of Fc γ R expression was clearly demonstrated in a model independent of a need for IgE-Fc ϵ RI-mast cells. Fc γ R expression was linked in this approach to APC function with full reconstitution of all responses when APCs expressing Fc γ R were injected into

FcR $\gamma^{-/-}$ recipients before sensitization; even when administered after sensitization and before challenge, APCs expressing FcR γ were capable of reconstituting lung allergic responses, albeit to a lower level than when injected during sensitization. These findings identify the potential of targeting activating FcRs in the treatment of asthma.

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