

IgE Influences the Number and Function of Mature Mast Cells, but Not Progenitor Recruitment in Allergic Pulmonary Inflammation¹

Clinton B. Mathias,^{*‡} Eva-Jasmin Freyschmidt,^{*‡} Benjamin Caplan,^{*} Tatiana Jones,[†] Dimitri Poddighe,^{*‡} Wei Xing,[†] Krista L. Harrison,^{*} Michael F. Gurish,^{†‡} and Hans C. Oettgen^{2*‡}

Studies performed using cultured cells indicate that IgE functions not only to trigger degranulation of mast cells following allergen exposure, but also to enhance their survival. Such an influence of IgE on mast cell homeostasis during allergic responses in vivo has not been established. In this study, we show that inhalation of *Aspergillus fumigatus* extract in mice induced a dramatic rise in IgE accompanied by an increase in airway mast cells. These had an activated phenotype with high levels of FcεRI. Plasma mast cell protease-1 was also increased, indicating an elevated systemic mast cell load. In addition, enhanced levels of IL-5 and eosinophils were observed in the airway. Both mast cell expansion and activation were markedly attenuated in IgE^{-/-} animals that are incapable of producing IgE in response to *A. fumigatus*. The recruitment of eosinophils to the airways was also reduced in IgE^{-/-} mice. Analyses of potential cellular targets of IgE revealed that IgE Abs are not required for the induction of mast cell progenitors in response to allergen, but rather act by sustaining the survival of mature mast cells. Our results identify an important role for IgE Abs in promoting mast cell expansion during allergic responses in vivo. *The Journal of Immunology*, 2009, 182: 2416–2424.

Asthma commonly affects individuals with atopy, a genetic predisposition to generate allergen-specific IgE Abs. The disease is characterized both by T cell-driven airway inflammation with eosinophil and mononuclear cell (MNC)³ infiltration of the airway mucosa and by superimposed acute exacerbations triggered by the interaction of IgE Abs with inhaled aeroallergens (1–4). Pedigree analyses reveal an association between IgE levels and disease activity in asthma (5–9). In allergic subjects, acute allergen-induced decreases in airflow are markedly blunted by IgE blockade with the anti-IgE mAb, omalizumab (10). Mast cells are present in the airways of patients with asthma (11–13), where they are optimally situated to trigger hypersensitivity responses upon exposure to inhaled environmental Ags. A correlation has been observed between numbers of mast cells in bronchial smooth muscle and asthma severity (14).

Although IgE Abs provide a critical Ag-specific activation function for mast cells in initiating both immediate hypersensitivity and late-phase responses, there is evidence that they also regulate mast cell survival and phenotype. Several recent studies using cultured bone marrow-derived mast cells (BMMC) have established that IgE can function as a survival factor for mast cells in an FcεRI-dependent fashion (15–18). In addition to enhancing BMMC survival, IgE Abs, even in the absence of Ag, have been shown to modulate their phenotype, increasing levels of transcripts for IL-6, IL-4, TNF-α, and IL-13, as well as secretion of IL-6 protein. We have observed that the splenic mastocytosis induced by infestation with the parasite, *Trichinella spiralis*, is IgE dependent (19), and Kitaoura et al. (20) have shown that IgE can enhance mast cell responses in mice injected with an IgE-secreting hybridoma.

These observations led us to hypothesize that IgE Abs produced in the course of an allergic response might serve not only to arm mast cells for allergen-triggered mediator release, but also to sustain mast cell expansion, promote FcεRI expression, and induce an activated phenotype. Such an IgE-driven amplification of the mast cell population and activation state might provide an increased tissue reservoir both of mediators of immediate hypersensitivity and of the cytokines and chemokines capable of initiating the recruitment of inflammatory cells in chronic allergic disease. To test this hypothesis and assess the role of IgE Abs in airway mast cell responses during allergen exposure, we chose to use a model of airway inflammation in which mice are repeatedly exposed to an aqueous extract of *Aspergillus fumigatus* (Af), resulting in the production of levels of IgE higher than elicited in OVA models. Our analyses of the Af responses of IgE^{-/-} mice previously established that intense airway inflammation and bronchial hyperresponsiveness (BHR) can arise in the absence of IgE (21). However, neither the effect of Af inhalation on airway mast cell numbers nor the role of IgE Abs in regulating mast cell homeostasis in this allergic disease model has been studied before.

^{*}Division of Immunology, Children's Hospital, Boston, MA 02115; [†]Division of Rheumatology, Allergy, and Immunology, Brigham and Women's Hospital, Boston, MA 02115; and [‡]Harvard Medical School, Boston, MA 02115

Received for publication May 14, 2008. Accepted for publication December 13, 2008.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by the following National Institutes of Health grants: National Institute of Allergy and Infectious Diseases Grants R01-AI054471 (to H.C.O.) and P01 AI 031599 (to M.F.G.). This work was also supported by a research grant from Novartis Pharmaceuticals.

² Address correspondence and reprint requests to Dr. Hans C. Oettgen, Division of Immunology, Children's Hospital Boston, 300 Longwood Avenue, Boston, MA 02115. E-mail address: hans.oettgen@childrens.harvard.edu

³ Abbreviations used in this paper: MNC, mononuclear cell; Af, *Aspergillus fumigatus*; BAL, bronchoalveolar lavage; BHR, bronchial hyperresponsiveness; BMMC, bone marrow-derived mast cell; Lin, Lineage; MCp, mast cell progenitor; NS, normal saline; SCF, stem cell factor; SM, staining medium; WT, wild type.

We found that mice subjected to repeated inhalation of *Af* displayed a vigorous IgE response accompanied by a robust expansion of mast cells in the trachea and mainstem bronchi. These mast cells expressed higher levels of Fc ϵ RI and produced elevated levels of IL-5 compared with those from saline-treated control animals. In contrast, IgE^{-/-} mice, which were incapable of generating an IgE response to *Af*, exhibited neither the increased numbers of mature mast cells nor the increased Fc ϵ RI density or IL-5 expression observed in wild-type (WT) animals. The expansion of mast cells in *Af*-treated WT mice was associated with enhanced levels of IL-5 and a more vigorous recruitment of eosinophils in the airways of WT than IgE^{-/-} mice.

We considered it likely that IgE Abs might exert their effect on airway mast cell numbers at the level of mature mast cells (which express the IgE receptor, Fc ϵ RI) rather than on receptor-negative progenitors. This hypothesis was supported by our observation that mast cell progenitors (MCP) were recruited to the inflamed airways of both WT and IgE^{-/-} mice following *Af* exposure, but that survival of adoptively transferred mature mast cells was preferentially sustained in mice with high IgE levels. These findings identify important novel roles for IgE Abs in the regulation of mast cell homeostasis and phenotype during allergic responses *in vivo*.

Materials and Methods

Reagents and mice

A sterile aqueous extract of *Af* (1:10 w/v at 87,000 protein nitrogen units/ml) was obtained from Greer Laboratories. IgE^{-/-} mice (22) were bred onto a BALB/c background (10 generations). WT BALB/c mice were purchased from Taconic Farms. Mice were housed in a specific pathogen-free environment and were 6–12 wk old. All experiments were conducted in accordance with the Institutional Animal Care and Use Committee policies and procedures of Children's and Brigham and Women's Hospitals.

Sensitization of mice

Mice were lightly anesthetized by isoflurane inhalation, and 50 μ l of *Aspergillus* Ag or saline was applied to the nares. Mice were immunized three times per week for 3 wk and were studied 12–24 h after the final dose.

Histological analysis

Airway mast cells were enumerated by microscopic examination of sections of paraffin-embedded airway tissue. Briefly, tracheae and main-stem bronchi were fixed in 10% buffered formalin and embedded in paraffin. Tissue sections were stained with toluidine blue and chloroacetate esterase. Mast cells were counted in four to five complete cross-sections of trachea, bronchus, or spleen, as specified in the figure labels. The numbers of mast cells were calibrated to unit length of epithelium (airway) or tissue area (spleen).

Collection of specimens

For bronchoalveolar lavage (BAL), 1 ml of staining medium (SM) consisting of HBSS supplemented with 10 mM HEPES buffer (pH 7.2) and 2% newborn calf serum was infused into the trachea and then retrieved. To obtain lung cell suspensions for flow cytometry, whole lungs were minced and treated with 1.3 mM EDTA in HBSS at 37°C for 30 min with shaking, followed by digestion with 100 U/ml collagenase (Life Technologies) in RPMI 1640 containing Ca²⁺ and Mg²⁺ and 5% FCS, at 37°C for 1 h. Released cells were mashed through a cell strainer (BD Biosciences) and washed with PBS. Erythrocytes were lysed by hypotonic shock, and cells were resuspended in SM. Total cells were enumerated, and viability was assessed by trypan blue exclusion. The viable cells (propidium iodide negative) in these preparations contained 80–85% CD45⁺ cells, and the majority of these (75–85%) resided in the MNC gate based on forward and side scatter analysis.

BAL cytology

BAL fluid was cytocentrifuged onto slides, and differential cellular analysis was assessed by light microscopy after staining with DiffQuik (Baxter).

Flow cytometry

Total cells from lung, spleen, BAL, and peritoneal lavage were analyzed by flow cytometry. Mast cells were stained as follows: cells were incubated with Fc block (anti-CD16/CD32) for 10 min on ice to prevent any non-specific binding via the FcR, followed by incubation with 10 μ g/ml anti-DNP IgE for 45 min at 4°C. Cells were washed and then incubated with various combinations of anti-c-Kit, anti-IgE, and mAbs specific for lineage markers, including CD3, CD4, CD8, CD45R, CD11b, and Gr-1 for 30 min on ice. MCP were similarly identified by using a mixture of Abs to CD45, CD34, and β_7 integrin while excluding lineage markers mentioned above. All mAbs were conjugated with either FITC, PE, allophycocyanin, PE-Cy5, or allophycocyanin-Cy7, and were purchased from eBioscience and BD Biosciences. Cells were washed and resuspended in SM containing propidium iodide to differentiate between live and dead cells. Cells were analyzed using FACSCalibur and FACS Canto flow cytometers, and data were processed using either CellQuest (BD Biosciences) or FlowJo (Tree Star) software.

Murine mast cell protease 1, IL-5, and total IgE ELISA

Murine mast cell protease 1 ELISA was performed on serum samples obtained after 3 wk of treatment with *Af* or normal saline (NS) using a kit (Moredun Scientific), according to the manufacturer's instructions. IL-5 ELISA was performed on BAL supernatants using the BD Biosciences murine IL-5 ELISA set. Total IgE levels in the sera were quantified by sandwich ELISA, as previously described (23).

Intracellular cytokine staining

Peritoneal lavage cells from WT or IgE^{-/-} mice were incubated with brefeldin A for 5 h at 37°C. Cells were then washed, incubated with anti-DNP IgE (20 μ g/ml) at 4°C, and stained for the surface markers c-Kit and IgE. They were then washed, permeabilized, and fixed using the Cytofix/Cytoperm buffer from BD Biosciences, and incubated with an Ab to IL-5. Cells were washed again, resuspended in fixative, and read using a FACSCalibur.

Culture of BMMC

BMMC were generated, as previously described (24). Briefly, marrow was obtained from the femurs and tibiae of naive WT mice, and cultured with 10 ng/ml IL-3 and 10 ng/ml stem cell factor (SCF; Peprotech) for 4–6 wk. Harvested BMMC were >95% positive for c-Kit and Fc ϵ RI by flow cytometry, and had characteristic appearance of mast cells on microscopic examination of toluidine blue-stained cytospun specimens.

Quantitative PCR analysis of IL-5

Total RNA was extracted from BMMC using TRIzol reagent (Invitrogen) following the manufacturer's instructions. cDNA was generated with iScript cDNA synthesis kit (Bio-Rad). Quantitative real-time PCR was done using Taqman Gene Expression Assay probe, Taqman PCR master mix, and the ABI Prism 7300 sequence detection system, all from Applied Biosystems. Relative expression of IL-5 was calculated in comparison with a reference gene transcript, β_2 -microglobulin, using the method described by Pfaffl (25).

MCp assay

The MCP assay was performed, as previously described (24, 26). Briefly, a single-cell suspension of lung MNCs was obtained from saline- and *Af*-treated WT and IgE^{-/-} mice. The cells were serially diluted 2-fold, and 100 μ l of each dilution was plated in 96-well flat-bottom microtiter plates in 24 replicates. Cells were cultured with 100 μ l of 10⁵ naive irradiated splenic feeder cells and recombinant cytokines, IL-3 (20 ng/ml) and SCF (100 ng/ml) at 37°C for 12–14 days. Wells containing mast cell colonies were enumerated using an inverted microscope. The MCP concentration was expressed as the number of MCP obtained per million MNCs. The total number of MCP/lung was derived by multiplying the concentration of MCP by the MNC yield per lung for individual mice, or divided by the total number of lungs when MNCs were pooled from more than one mouse.

CFSE labeling and transfer of mast cells

BMMC were treated with 5 μ M CFSE/DMSO in PBS and incubated at 37°C for 5 min without shaking. CFSE labeling was quenched by addition of ice-cold medium. CFSE labeling was confirmed to be greater than 99% before adoptive transfer. Approximately 2.0 $\times$ 10⁶ cells were injected i.p.

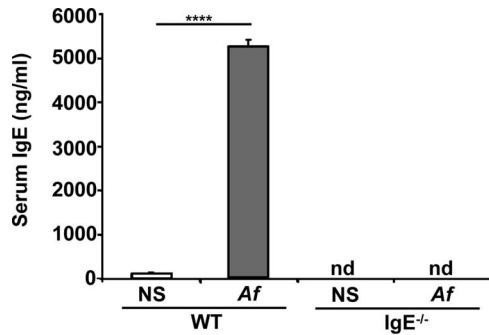


FIGURE 1. Serum IgE levels are elevated in *Af*-treated WT, but not IgE^{-/-} mice. Total serum IgE was determined by sandwich ELISA in WT or IgE^{-/-} mice treated with *Af* or NS for 3 wk. Eight to 10 mice were analyzed in each group. nd = None detected; ****, $p < 0.0001$.

into WT and IgE^{-/-} mice, which had been subjected to the 3-wk *Af* regimen, followed by 1 wk of rest. Similarly, a single mouse in each group received equivalent numbers of unlabeled cells to compare background signal in the FL-1 channel. Following transfer, five experimental mice from each group were sacrificed on days 1, 2, 3, and 6, and peritoneal cells were examined for the presence of CFSE⁺c-Kit⁺FcεRI⁺ cells by flow cytometry. Total numbers of positive cells were enumerated as a fraction of the total peritoneal cellular inflammation.

BrdU incorporation and transfer of mast cells

During the final week of BMMC generation, BMMC were cultured in the presence of 10 μM BrdU (BD Biosciences). BrdU⁺ WT BMMC were subsequently harvested, and $\sim 1 \times 10^6$ cells were injected i.p. into WT and IgE^{-/-} mice, which had been subjected to the 3-wk *Af* regimen, followed by 1 wk of rest. Six days following transfer of BrdU⁺ mast cells, mice were sacrificed and peritoneal cells were examined for the presence of BrdU⁺ cells, expressing c-Kit and FcεRI by flow cytometry using the BrdU Detection Kit (BD Biosciences). Background fluorescence was assessed by similarly injecting *Af*-treated mice with WT BMMC, which had been cultured in the absence of BrdU.

Determination of apoptosis in transferred mast cells

Six days after transfer, transferred mast cells were isolated from the peritoneum and examined for the induction of apoptosis using annexin V staining (BD Biosciences). Cells were stained with annexin V, and the total numbers of annexin V-positive mast cells were enumerated.

Statistical analysis

Differences in values for various experimental groups were examined for significance using two-tailed Student's *t* test. All data are shown as the mean response $\pm$ SEM.

Results

IgE Abs support airway mast cell expansion following *Af* inhalation

Mice were treated either with saline or *Af* intranasally over a period of 3 wk, resulting in a vigorous IgE response in WT, but not IgE^{-/-} animals (Fig. 1). A dramatic expansion of toluidine blue- and chloroacetate esterase-positive spindle-shaped mast cells containing heterochromatic granules was seen in the trachea and mainstem bronchi of WT mice after *Af* exposure (Fig. 2A). Enumeration of mast cells in bronchi revealed increases from <3 cells/mm to ~ 10 cells/mm, with a similar increase from 1 cell/mm to >5 cells/mm in tracheae (Fig. 2B). Numerous degranulating cells were evident. Intraepithelial mast cells were also seen in some sections, and many of the mast cells were located around tracheal smooth muscle. In contrast, *Af* treatment did not significantly alter mast cell numbers in the airways of IgE^{-/-} mice. Mast cell expansion was not restricted to the tissue site of initial allergen encounter. In the spleens of

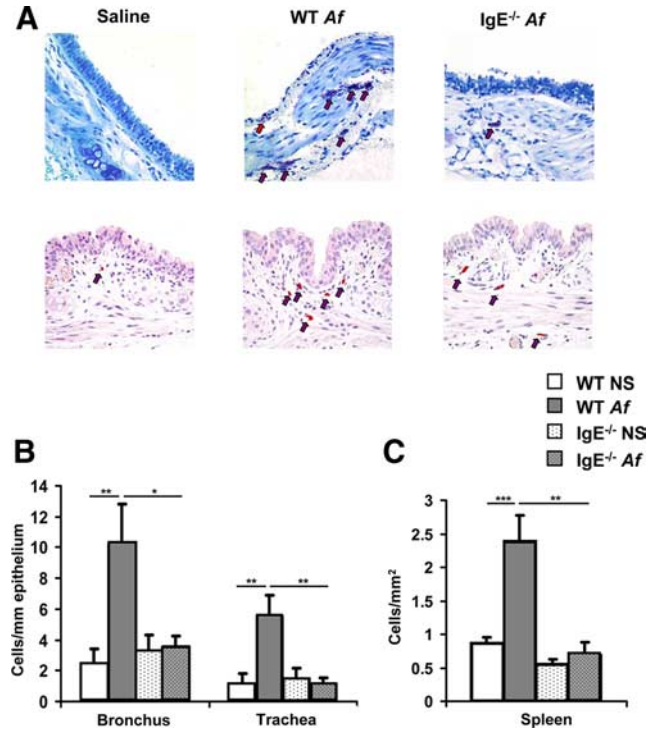


FIGURE 2. Expansion of mast cells is induced in the trachea, bronchus, and spleen of *Af*-treated WT, but not IgE^{-/-} mice. WT and IgE^{-/-} mice were treated intranasally with either *Af* or NS for 3 wk, and mast cells were enumerated. A, Toluidine blue (upper panels)- and chloroacetate esterase (lower panels)-positive mast cells in representative cross-sections of trachea. Mast cells indicated by arrows (red arrow for an intraepithelial mast cell). B and C, Toluidine blue-positive mast cells in the main-stem bronchi, tracheae, and spleens of *Af*-treated animals. $n = 5$ mice per group; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Af-treated mice, mast cell numbers were also ~ 3 -fold higher in WT mice than in IgE^{-/-} animals (Fig. 2C).

The requirement for IgE in mast cell expansion was further assessed by flow cytometric analysis of BAL fluid. Mast cells are typically identified on the basis of expression of c-Kit and FcεRI. However, as we and others have observed, the levels of FcεRI are so diminished on mast cells from IgE^{-/-} mice that enumeration of mast cells cannot be reliably performed in IgE^{-/-} mice using FcεRI expression (27, 28). To circumvent this technical challenge, we identified mast cells on the basis of expression of c-Kit and the absence of lineage markers for T (CD3, CD4, CD8) and B (B220) lymphocytes as well as granulocytes (Gr-1), as we have reported (29). We established that this staining specifically identified mast cells by showing that the Lineage⁻ (Lin) c-Kit⁺ cells obtained from WT mice indeed uniformly expressed FcεRI (Fig. 3A). CD34 was considered as an alternative mast cell marker. However, we and others have observed down-regulation of CD34 on mucosal mast cells under certain circumstances, precluding reliance upon this Ag for their enumeration. Analysis of the BAL of *Af*-treated animals by this flow cytometric approach revealed that recruitment of mast cells to the airway of IgE^{-/-} mice (770 ± 100 cells/ml) was significantly blunted compared with WT controls (2090 ± 200 cells/ml) (Fig. 3B). Taken together with the results of our histologic enumeration of mast cells, these results provide strong evidence that *Af*-induced mast cell expansion in the airways is enhanced in the presence of IgE Abs.

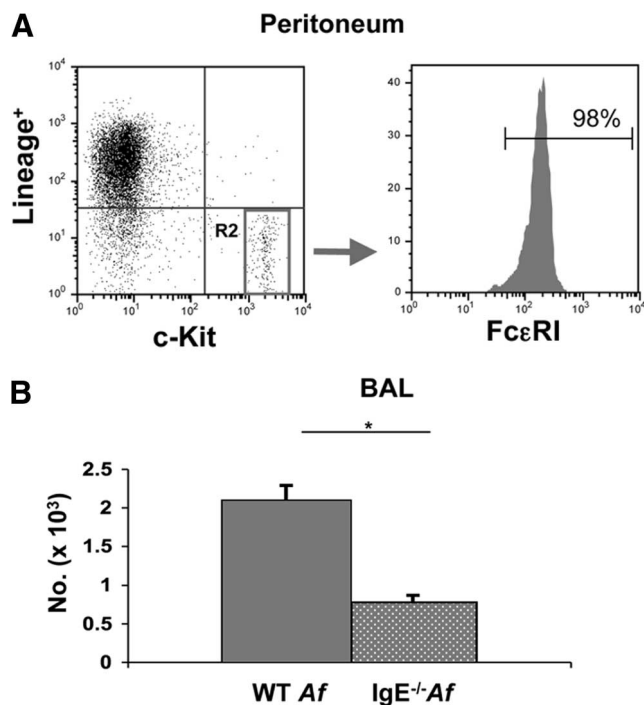


FIGURE 3. Airway mast cells, enumerated by flow cytometry, are increased in WT vs IgE^{-/-} Af-treated mice. Peritoneal lavage and BAL fluid from Af-treated mice were examined by flow cytometry. *A*, Confirmation of the mast cell phenotype of Lin⁻ c-Kit⁺ peritoneal cells. Cells were stained with lineage-specific Abs (CD3, B220, CD4, CD8, Gr-1) and anti-c-Kit. Cells residing in the Lin⁻ c-Kit⁺ gate, (R2), were analyzed for FcεRI expression. *B*, Lin⁻ c-Kit⁺ mast cells are increased in the BAL of Af-treated WT mice. Cells from control NS mice could not be analyzed because inadequate numbers were present in the BAL. *n* = 5 mice per group; *, *p* < 0.05.

The expression of FcεRI on mast cells during Af-induced allergic disease is regulated by IgE

We have previously shown that IgE acts as a positive regulator of its high-affinity receptor, FcεRI, on mast cells (28). FcεRI-mediated signaling has been shown to be critical for the *in vitro* effects of IgE on the survival of cultured mast cells (15, 16). Therefore, we examined the expression of FcεRI on peritoneal and lung mast cells in Af-treated mice. FcεRI was significantly up-regulated on both peritoneal (Fig. 4*A*) and lung (Fig. 4*B*) mast cells in WT mice following Af exposure (FcεRI median fluorescence intensity increased from 133 ± 40 to 663 ± 36 on lung cells). In contrast, FcεRI expression was unchanged following Af treatment in IgE^{-/-} mice. As we have previously observed, FcεRI levels were lower in IgE^{-/-} than WT mast cells even at physiologic baseline IgE levels. The intensity of staining was lower on both peritoneal and lung mast cells from IgE^{-/-} saline control mice than in cells from their WT counterparts.

Murine mast cell protease 1 levels are increased during Af-induced allergic inflammation

The release of mast cell proteases during allergic inflammation provides a serum marker of mast cell activation (30, 31). We assessed the levels of serum murine mast cell protease 1 in WT and IgE^{-/-} mice after Af treatment and found that serum murine mast cell protease 1 was undetectable in the serum of control mice (the detection limit of the murine mast cell protease 1 ELISA is 0.25 ng/ml), but rose significantly following Af treatment (399 ± 20 ng/ml). This increase was significantly blunted in mice lacking

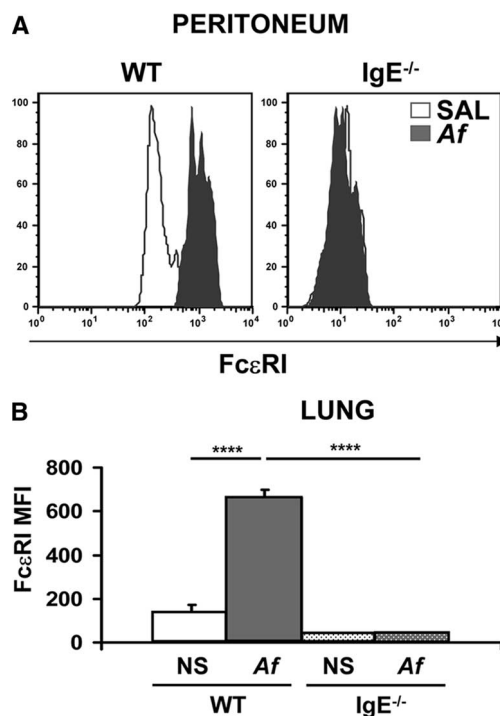


FIGURE 4. FcεRI expression on mast cells from Af-treated mice is increased in the presence of IgE. Peritoneal and lung cells were isolated from NS- or Af-treated mice, and mast cells were examined by flow cytometry. *A*, FcεRI staining on peritoneal mast cells from Af-treated (solid histograms) WT and IgE^{-/-} mice is shown overlaid on expression in NS control animals (unfilled histograms) of the same genotypes. The levels of FcεRI were assessed by staining with anti-DNP IgE, followed by anti-IgE. *B*, Median fluorescence intensity of FcεRI expression on c-Kit⁺ cells from collagenase-digested lung tissue in NS- or Af-treated WT and IgE^{-/-} mice. *n* = 5 mice per group; ****, *p* < 0.0001.

IgE, which displayed only 28% (111 ± 36 ng/ml) of the WT murine mast cell protease 1 levels (Fig. 5). These data confirm our histologic and flow cytometric evidence that mast cell expansion in the setting of allergen exposure is supported by IgE Abs.

IL-5 production by mast cells is enhanced by IgE Abs

Allergic inflammation is characterized by enhanced production of Th2 cytokines. One of these, IL-5, is important both for eosinophilopoiesis and eosinophil recruitment. We observed significantly

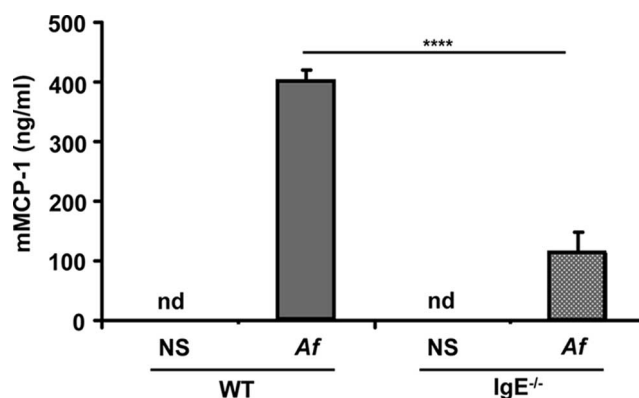


FIGURE 5. Af-induced increases in murine mast cell protease 1 levels in Af-treated mice are enhanced in the presence of IgE. Sera were isolated from NS- or Af-treated WT and IgE^{-/-} mice after 3 wk of treatment, and murine mast cell protease 1 levels were quantified by ELISA. *n* = 5 mice per group. nd = None detected; ****, *p* < 0.0001.

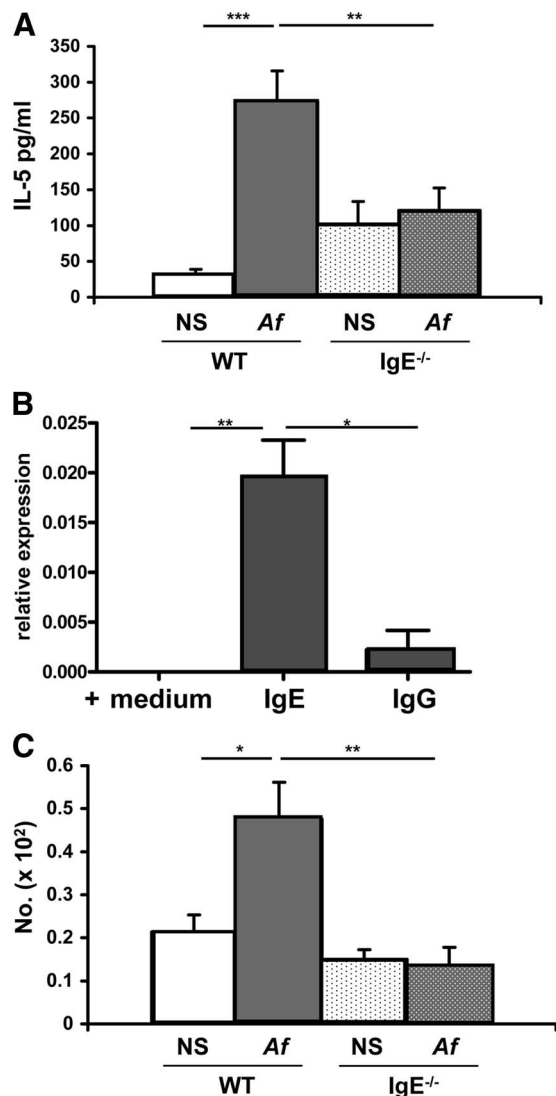


FIGURE 6. BAL IL-5 levels and IL-5 expression by cultured mast cells and mast cells in vivo are regulated by IgE Abs. *A*, BAL fluid was obtained from NS- or Af-treated WT and IgE^{-/-} mice, and the levels of IL-5 were determined by sandwich ELISA. *B*, BMMC were cultured in the presence of medium or 10 μ g/ml IgE for 60 min. IL-5 mRNA was measured using real-time quantitative PCR. IgG was used as a control. *C*, Peritoneal cells were isolated from NS- or Af-treated WT and IgE^{-/-} mice, and the number of IL-5-positive cells was assessed by intracellular staining. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

increased levels of IL-5 in the airways of WT animals as compared with their IgE^{-/-} counterparts (Fig. 6A). In contrast, no difference was observed in the levels of IL-13 between the two groups of mice (data not shown). We considered airway mast cells as a potential source of IL-5 and examined the effects of IgE Abs on their capacity to produce this cytokine. Mast cells cultured in the presence of IgE have previously been shown to produce IL-6, TNF- α , IL-4, and IL-13 (16). We observed that BMMC could similarly be induced to produce mRNA for IL-5 when cultured with IgE (Fig. 6B). To establish whether IgE Ab levels similarly modulate mast cell IL-5 production in vivo, we performed intracellular IL-5 staining on freshly isolated mast cells from WT and IgE^{-/-} mice. Because it was not possible to isolate sufficient numbers of pulmonary Lin⁻, c-Kit⁺ mast cells for this analysis, we examined more readily available peritoneal mast cells that, we reasoned, had been exposed to the same ambient IgE levels. Intracellular IL-5 staining

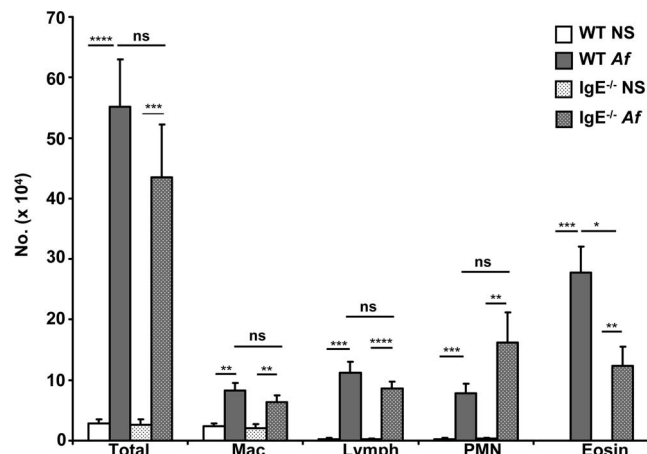


FIGURE 7. IgE Abs regulate the pulmonary cellular infiltrate elicited by allergen inhalation. Differential cell counts were performed on Wright-Giemsa-stained cytocentrifuged BAL specimens from WT and IgE^{-/-} mice treated with Af or NS. Percentages of macrophages (Mac), lymphocytes (lymph), polymorphonuclear neutrophils (PMN), and eosinophils (eosin) were enumerated in individual samples and are depicted as a fraction of the total cell count. $n = 5$ mice per group. ns, Not significant. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

of peritoneal mast cells revealed that significantly more mast cells from Af-treated WT mice stained IL-5 positive than from NS controls, and this induction was observed only in WT, but not IgE^{-/-} mice (Fig. 6C). These findings indicate that IgE Ab levels are important in regulating mast cell IL-5 production.

IgE-induced mast cell expansion and IL-5 production are accompanied by increased airway eosinophilia in WT mice

Activated mast cells produce a number of cytokines and chemokines capable of acting upon vascular endothelium and trafficking leukocytes to orchestrate an inflammatory cell infiltrate. Given the increase in mast cells in the airways of allergen-treated animals, as well as our observation of IgE effects on mast cell IL-5 production, we considered the possibility that cellular infiltration into the allergen-exposed airway might be modulated by IgE. BAL fluid of Af-treated WT and IgE^{-/-} mice was subjected to cytologic analysis to characterize infiltrating cell populations. A very dramatic increase in airway cells was induced by Af treatment in both WT and IgE^{-/-} mice (Fig. 7), consistent with our previous observation that allergen-induced airway inflammation can arise independently of IgE Abs (21). As expected, the cellular profile included macrophages, lymphocytes, neutrophils, and eosinophils. Notably, WT and IgE^{-/-} mice differed with respect to recruitment of eosinophils to the airway. Although a significant eosinophil influx occurred both in the presence and absence of IgE, the BAL of IgE^{-/-} mice contained less than 50% of the number of eosinophils as did WT animals (Fig. 7). There appeared to be no other statistically significant differences in the cellular composition of WT vs IgE^{-/-} BAL. An apparent trend toward increased numbers of neutrophils was observed in the BAL of IgE^{-/-} mice, although not reaching statistical significance, suggesting that the decrease in eosinophils was accompanied by a parallel increase in neutrophils in the absence of IgE.

IgE is not required for the recruitment of MCP to the lungs of Af-challenged mice

Tissue mast cell expansion during immune responses to parasites and allergens is thought to depend on the recruitment of mast cell

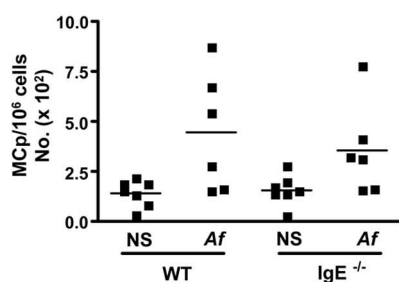
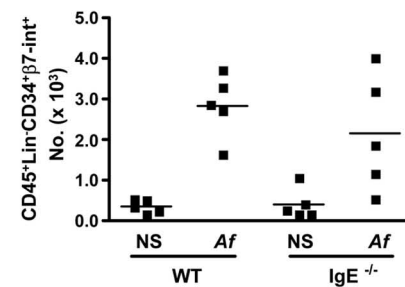
A Limiting Dilution Mast Cell Progenitor Assay**B** Phenotypic Analysis of Mast Cell Progenitors

FIGURE 8. MCP recruitment to the airways of *Af*-treated mice occurs independently of ambient IgE levels. Lung MNCs were isolated from naive or *Af*-treated WT and IgE^{-/-} mice, and MCP were enumerated by limiting dilution culture of IL-3/SCF cultures of lung MNCs, as described in *Materials and Methods*. *A*, MCP/million lung MNCs and total numbers of lung MCP are shown. *B*, CD45⁺Lin⁻CD34⁺β7-int⁺ lung MCP as assessed by flow cytometry.

precursors (MCP) to sites of inflammation (24, 32–34). To examine whether the expansion of mast cells in our model was a consequence of increased recruitment of MCP to the airways following allergen exposure, we performed limiting dilution analysis for lung MCP (Fig. 8*A*). Using this approach, we found that the lungs of saline-treated mice had fewer than 250 MCP per million cells and less than 1000 total MCP per mouse. However, *Af* treatment induced robust recruitment of MCP to the lungs of both WT (4772 total) and IgE^{-/-} (3976 total) mice, indicating that the recruitment of mast cell precursors during allergic inflammation occurs by IgE-independent mechanisms.

To confirm this observation, we used a flow cytometric approach that we have previously shown to enumerate tissue MCP (24). Tissue MCP are identified as CD45⁺, Lin⁻ (CD3, CD4, CD8, B220, CD11b, Gr-1)⁻, CD34⁺, and β₇ integrin⁺. Consistent with the limiting dilution assay, examination of lung MNCs for the presence of cells with the antigenic phenotype of MCP showed that allergen exposure led to a strong recruitment of MCP and that comparable numbers of MCP were present in the lungs of *Af*-treated WT and IgE^{-/-} mice (Fig. 8*B*). The functional and flow cytometric methods for MCP enumeration were quite consistent with each other, indicating similar numbers of cells. These findings therefore suggest that IgE Abs are not required for the recruitment of mast cell precursors to the airways, leading us to speculate that the IgE effect most likely occurs at the level of the mature, FcεRI-expressing mast cell.

IgE is required for the survival of differentiated mast cells in vivo

Because IgE Abs appeared not to be necessary for the recruitment of MCP during *Af*-induced allergic inflammation, we tested the hypothesis that they might instead enhance the survival of differentiated mast cells *in vivo*, just as they have previously been shown to support survival of cultured cells (15, 16). BMDC were labeled with CFSE and adoptively transferred into the peritoneum of WT and IgE^{-/-} mice that had been treated with *Af* to elicit IgE responses. Enumeration of transferred CFSE⁺c-Kit⁺FcεRI⁺ mast cells in the peritoneal fluid of recipient mice showed a gradual decline over a period of 6 days in both WT and IgE^{-/-} mice (Fig. 9*A*). Although initial mast cell recovery at day 1 was similar in WT and IgE^{-/-} animals, consistent with comparable levels of reconstitution, mast cell numbers were significantly lower in IgE^{-/-} mice on both days 3 and 6 after adoptive transfer, suggesting that IgE Abs enhanced survival of mast cells *in vivo* (Fig. 9*A*). No dilution of the CFSE signal was evident during the 6-day period of analysis (data not shown), indicating that IgE sustains mast cell survival rather than inducing mast cell proliferation.

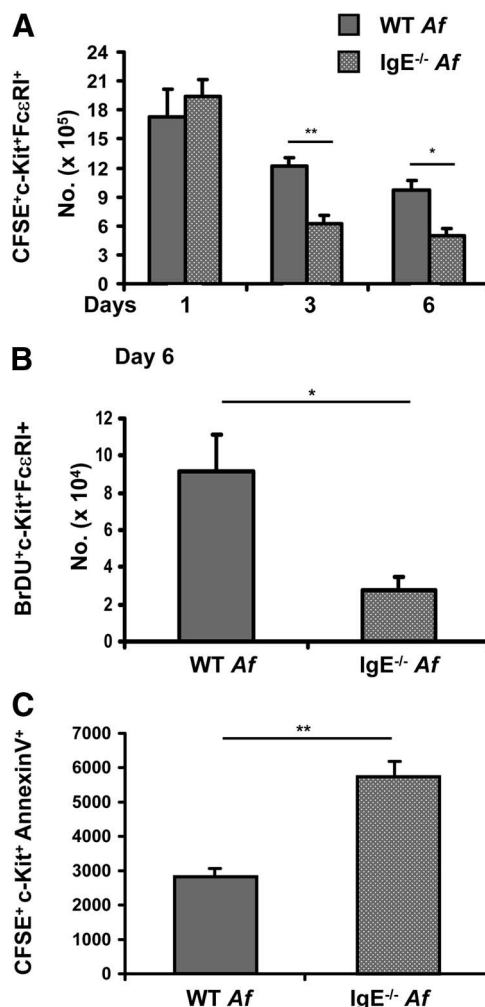


FIGURE 9. IgE supports the survival of differentiated mast cells *in vivo* and provides an antiapoptotic signal. *A*, WT and IgE^{-/-} mice were treated with *Af*. CFSE-labeled BMDC were injected (2×10^6 cells) into the peritoneal cavities of individual mice. Peritoneal cells were recovered 1, 3, and 6 days later, and CFSE⁺c-Kit⁺FcεRI⁺ mast cells were enumerated. Mice that received unlabeled BMDC served as controls. *n* = 5 mice per group. *, *p* < 0.05. *B*, BrdU-labeled BMDC (1×10^6 cells) were injected into the peritoneum of *Af*-treated, WT, and IgE^{-/-} mice. Six days after transfer, c-Kit⁺BrdU⁺ and FcεRI⁺ cells were enumerated by flow cytometry. *n* = 4 mice per group. *C*, CFSE-labeled BMDC were injected into the peritoneal cavities of *Af*-treated WT and IgE^{-/-} mice. Six days later, transferred BMDC were recovered and apoptosis was assessed by determining surface levels of annexin V by flow cytometry. Total numbers of CFSE⁺c-Kit⁺annexinV⁺ cells in the peritoneum are shown. *, *p* < 0.05; **, *p* < 0.01.

To confirm the effects of IgE on cell survival suggested by the CFSE experiments, we similarly examined the recovery of BrdU-labeled mast cells after adoptive transfer into the peritoneum of *Af*-treated WT and IgE^{-/-} mice. Six days after transfer, ~3-fold more BrdU⁺ mast cells were recovered from the peritoneum of *Af*-treated WT mice than from their IgE^{-/-} counterparts (Fig. 9B). The recovered mast cells expressed both c-Kit and FcεRI, as expected.

The relative paucity of transferred mast cells recovered from the peritoneal cavities of IgE^{-/-} vs WT mice suggested the possibility of IgE delivering antiapoptotic signals, as has been observed in cultured mast cells (15, 16). The possibility of a similar antiapoptotic effect of IgE in vivo was examined by assessing surface levels of annexin V (35) on transferred mast cells, the same method that had been used by Asai et al. (15) to assess apoptosis levels in cultured mast cells exposed to IgE. Significantly higher levels of annexin V were detected in CFSE⁺ transferred mast cells recovered from *Af*-treated IgE^{-/-} mice as compared with WT animals (Fig. 9C), mirroring the survival data shown in Fig. 9, A and B. Taken together, these data indicate that IgE Abs regulate the survival of mature mast cells in the setting of in vivo allergic responses.

Discussion

Mast cells armed with specific IgE Abs and residing in the mucosa serve as airway sentinels, sensing and responding to inhaled Ags. In patients with asthma, the IgE-mediated activation of these cells following aeroallergen exposure induces release of vasoactive and smooth muscle-constricting mediators that trigger acute airflow obstruction, as well as the production of bioactive lipids, cytokines, and chemokines that act in concert to set the stage for eventual cellular influx and allergic airway inflammation. Our results provide strong evidence that IgE Abs not only serve as allergen sensors for airway mast cells, but also function as critical regulators of their survival and phenotype. Thus, the cell-sustaining properties of IgE Abs for cultured mast cells recently reported by a number of groups are important during IgE-inducing allergic responses in vivo as well.

IgE effects on mast cell homeostasis are not restricted to the allergic state; we have reported that the splenic mastocytosis that occurs during infestation with the parasite, *Trichinella spiralis*, is IgE dependent (19), and Kitaura and colleagues (20) have shown that IgE can regulate mast cell homeostasis in mice injected with an IgE-secreting hybridoma.

The presence of mast cells in bronchial mucosa and smooth muscle is associated with asthma in humans; patients with other forms of bronchial inflammation do not harbor mast cells at these sites (reviewed in Refs. 36 and 37). Mast cell density in the smooth muscle is correlated with responsiveness to methacholine (14). Mast cells also infiltrate mucous glands, where they regulate secretion and can additionally be found in the bronchoalveolar space and around airway blood vessels. Chronic asthma is characterized by progressive irreversible airflow obstruction believed to be a consequence of airway remodeling, tissue changes in the airway that include fibrosis and angiogenesis. Because mast cells are closely associated with airway blood vessels and produce angiogenic factors including histamine, proteases, TGF-β, and vascular endothelial growth factor, they may provide critical signals for these long-term changes. In the present study, we did not observe differences in subepithelial collagen deposition or total lung collagen (data not shown) between IgE^{-/-} vs WT animals, but it is likely that the 3-wk *Af* protocol does not really recapitulate the years of chronic allergen exposure experienced by patients.

Several groups have used murine models to assess the role of mast cells and IgE Abs in allergen-induced airway inflammation. An emerging theme from studies is that, under conditions of strong allergenic stimulation, chronic airway inflammation and BHR can arise completely independently of IgE and mast cells, but IgE Abs are important for acute responses to allergen inhalation. When less intense sensitization protocols are used, a contributory role for IgE and mast cells can be discerned even in the induction of chronic inflammation. It appears that, as has been observed in other systems, IgE and mast cells may serve as amplifiers of the inflammatory response (17, 38, 39). Our own studies with IgE^{-/-} mice along with the work of others has shown that airway inflammation and BHR can be elicited in allergen-challenged mice even in the absence of IgE or other Abs (21, 40). Similar findings have been reported regarding mast cells. Using a standard protocol in which mice are sensitized to OVA i.p. and then challenged by inhalation, Takeda and colleagues (41) found that airway inflammation and BHR were the same in WT and W/W^v mice. Kobayashi et al. (42) also observed no defect in eosinophil recruitment and airway inflammation in W/W^v mice, but did see a mast cell effect on BHR. The groups of Galli and Broide (43, 44) have established conditions under which protocols for OVA sensitization of the airways could be modified, using repetitive exposure, to induce mast cell expansion, thus allowing them to define significant contributions of these cells to the allergen-induced eosinophil-predominant inflammation and BHR. Studies by Mayr et al. (45), using such a chronic OVA protocol, suggested that the mast cell contribution to airway inflammation is IgE dependent, and subsequent experiments using adoptive reconstitution of W/W^v mice with BMMC from WT or FcεRI^{-/-} donors performed by Taube et al. (46) revealed a role for IgE in the airway mast cell response to allergen. Additional work by Yu et al. (44), who reconstituted W/W^v mice with BMMC from FcRγ^{+/+} and FcRγ^{-/-} donors, provided evidence for both IgE/IgG-dependent and IgE/IgG-independent mast cell contributions. Taken together, these reports suggest that mast cells and IgE Abs can serve as amplifiers of inflammation in the airway under physiologic allergen-limiting conditions.

The alum-free OVA models used in the studies reviewed above typically elicit IgE levels ~400–500 ng/ml (47). We have previously observed that inhalation alone (without i.p. priming) of aqueous extracts of *Af* can drive IgE responses that are as much as 10–100 times as high (21, 48). Because the central goal of our study was to examine the effects of IgE Ab levels on mast cell numbers, we chose to use this very robust IgE-inducing protocol. The *Af* model offered the additional advantage that it might more directly reflect the type of allergen exposure encountered by patients, namely a mixture of protein Ags and nonprotein fungal lipids and glycolipids with potential immunostimulatory adjuvant activities for allergic responses. Indeed, we found that repeated *Af* inhalation, as had been reported by others for repeated OVA inhalation, led to increased airway mast cell numbers in the airways and spleen. These increases were demonstrated using both histology and flow cytometry, and corroborated by serum murine mast cell protease 1 levels. We have previously published that IgE Abs are not required for the induction of airway inflammation and BHR in the *Af* model (21). In contrast, however, our current findings reveal a significant discrepancy between WT and IgE^{-/-} mice in terms of mast cell expansion, providing clear evidence that IgE Abs specifically regulate the homeostasis of this cell type. Furthermore, our present observations suggest that IgE also regulates eosinophilia in the *Af* model. In our earlier work, using mice on a 129SvEv background (BALB/c is the strain of the present study) and a different source of *Af*, we observed that IgE affected neither the recruitment of eosinophils to the BAL nor the induction of

BHR in *Af*-treated mice (21). In that study, the inflammatory response was markedly more intense, with IgE levels and total BAL eosinophil counts each about an order of magnitude higher than in the current investigation. We speculate that a combination of factors related to strain differences and *Af* extract potency may have induced such a strong inflammatory response in the former study as to override the amplifying eosinophil-enhancing effect of IgE and mast cells that we now observe.

Analyses of MCP frequency and mature mast cell survival in the *Af*-treated mice indicated that there was an intense recruitment of progenitors to the site of allergic airway inflammation regardless of the presence of IgE. Because these cells are at most weakly FcεRI positive, the absence of a requirement for IgE in their recruitment was anticipated (29). In contrast, our studies using cultured mature BMMC labeled with CFSE⁺ and BrdU⁺ revealed enhanced survival of differentiated mast cells in the peritoneum of *Af*-treated WT compared with IgE^{-/-} mice, supporting the hypothesis that IgE Abs directly regulate the homeostasis of mature mast cells during allergic responses in vivo.

Studies of IgE-mediated growth enhancement of cultured mast cells have not clearly elucidated a mechanism whereby IgE supports survival. To determine whether mast cell regulation by IgE might depend on the paracrine effects of other mast cell growth factors, we assayed the BAL of *Af*-treated WT and IgE^{-/-} mice for the presence of known mast cell stimulators, including IL-3, IL-4, and IL-9. We observed similar levels of IL-3 in both WT and IgE^{-/-} mice following *Af* inhalation and levels of IL-9 and IL-4 that were below the limits of detection (data not shown). These observations suggested to us that IgE supports mast cell survival in vivo independently of these cytokines. However, a recent report by Kohno et al. (49) suggested that IgE regulates the survival of cultured mast cells via production of IL-3. Preliminary studies in our laboratory indicate that mast cell numbers fail to increase in the lungs and BAL of IL-3^{-/-} mice despite elevated levels of IgE, suggesting that IL-3 may similarly be required for IgE-driven mast cell expansion in vivo (data not shown). Such a requirement for IL-3 has also been seen in the elicitation of mast cell responses during infestation with the nematode parasite, *Strongyloides venezuelensis* (50).

To determine whether, beyond enhancing mast cell numbers, IgE additionally regulates mast cell phenotype in vivo, we examined mast cells for the production of intracellular cytokines and for expression of FcεRI. Our finding that peritoneal mast cells from *Af*-treated WT mice made more IL-5 than mast cells in the IgE-free environment of IgE^{-/-} mice indicates that IgE can regulate the amplitude of mast cell mediator production. Similarly, the very low levels of FcεRI on mast cells from *Af*-treated IgE^{-/-} mice point to an important role for IgE Abs in regulating IgE receptor levels in vivo. There is good evidence that IgE regulation of FcεRI is operative in humans with allergic disease as well. In atopic cohorts, total serum IgE levels are correlated with basophil FcεRI density, and treatment with omalizumab has been found to cause diminished FcεRI expression on mast cells, basophils, and dendritic cells (51–54). It is tempting to speculate that induction of FcεRI by elevated IgE levels in atopic individuals might provide a positive feedback loop for supporting mast cell survival.

Several groups have reported diminished eosinophilic and MNC infiltration into the airways of mast cell-deficient mice repeatedly subjected to OVA inhalation (44, 45, 47). We have found that IgE-dependent allergen-induced mast cell expansion is also accompanied by decreased numbers of eosinophils in the airway, with an accompanying trend (statistically insignificant in our hands) toward increase in the numbers of neutrophils. Thus, the expansion and chronic activation of mast cells in the airways of

asthmatic patients along with their capacity to produce IL-5 and eosinophil-specific chemokines might serve to amplify the recruitment of eosinophils to the airways.

Production of IgE Abs is the hallmark of the atopic state. The association of IgE levels with asthma severity and the well-established function of IgE in driving immediate hypersensitivity reactions following allergen encounter have focused attention on this Ab isotype as a therapeutic target. Indeed, IgE inhibitors have been developed based on these aspects of IgE biology, and are, as expected, very effective in blocking acute allergen-induced airflow obstruction in asthmatics (10). Our findings provide novel insights into the role of IgE Abs in mast cell homeostasis and asthma pathogenesis, and suggest additional potential consequences of IgE blockade. Taken together with human and mouse data on the importance of mast cells in driving the asthmatic response, the results presented in this study suggest that modulation of IgE responses might provide benefits extending far beyond the inhibition of immediate hypersensitivity.

Acknowledgments

We thank Dr. Michiko Oyoshi for help with experiments.

Disclosures

The authors have no financial conflict of interest.

References

- Holgate, S. T. 2007. Epithelium dysfunction in asthma. *J. Allergy Clin. Immunol.* 120: 1233–1244; quiz 1245–1236.
- Larche, M., D. S. Robinson, and A. B. Kay. 2003. The role of T lymphocytes in the pathogenesis of asthma. *J. Allergy Clin. Immunol.* 111: 450–463; quiz 464.
- Meiler, F., M. Zimmermann, K. Blaser, C. A. Akdis, and M. Akdis. 2006. T-cell subsets in the pathogenesis of human asthma. *Curr. Allergy Asthma Rep.* 6: 91–96.
- Umetsu, D. T., and R. H. Dekruyff. 2006. Immune dysregulation in asthma. *Curr. Opin. Immunol.* 18: 727–732.
- Sears, M. R., B. Burrows, E. M. Flannery, G. P. Herbison, C. J. Hewitt, and M. D. Holdaway. 1991. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. *N. Engl. J. Med.* 325: 1067–1071.
- Van Herwerden, L., S. B. Harrap, Z. Y. Wong, M. J. Abramson, J. J. Kutin, A. B. Forbes, J. Raven, A. Lanigan, and E. H. Walters. 1995. Linkage of high-affinity IgE receptor gene with bronchial hyperreactivity, even in absence of atopy. *Lancet* 346: 1262–1265.
- Postma, D. S., E. R. Bleeker, P. J. Amelung, K. J. Holroyd, J. Xu, C. I. Panhuysen, D. A. Meyers, and R. C. Levitt. 1995. Genetic susceptibility to asthma: bronchial hyperresponsiveness coinherit with a major gene for atopy. *N. Engl. J. Med.* 333: 894–900.
- Barnes, K. C., J. D. Neely, D. L. Duffy, L. R. Freidhoff, D. R. Breazeale, C. Schou, R. P. Naidu, P. N. Levett, B. Renault, R. Kucherlapati, et al. 1996. Linkage of asthma and total serum IgE concentration to markers on chromosome 12q: evidence from Afro-Caribbean and Caucasian populations. *Genomics* 37: 41–50.
- Nickel, R., U. Wahn, N. Hizawa, N. Maestri, D. L. Duffy, K. C. Barnes, K. Beyer, J. Forster, R. Bergmann, F. Zepp, et al. 1997. Evidence for linkage of chromosome 12q15–q24.1 markers to high total serum IgE concentrations in children of the German Multicenter Allergy Study. *Genomics* 46: 159–162.
- Fahy, J. V., D. W. Cockcroft, L. P. Boulet, H. H. Wong, F. Deschesnes, E. E. Davis, J. Ruppel, J. Q. Su, and D. C. Adelman. 1999. Effect of aerosolized anti-IgE (E25) on airway responses to inhaled allergen in asthmatic subjects. *Am. J. Respir. Crit. Care Med.* 160: 1023–1027.
- Koshino, T., Y. Arai, Y. Miyamoto, Y. Sano, M. Itami, S. Teshima, K. Hirai, T. Takaishi, K. Ito, and Y. Morita. 1996. Airway basophil and mast cell density in patients with bronchial asthma: relationship to bronchial hyperresponsiveness. *J. Asthma* 33: 89–95.
- Koshino, T., Y. Arai, Y. Miyamoto, Y. Sano, T. Takaishi, K. Hirai, K. Ito, and Y. Morita. 1995. Mast cell and basophil number in the airway correlate with the bronchial responsiveness of asthmatics. *Int. Arch. Allergy Immunol.* 107: 378–379.
- Gibson, P. G., N. Salto, and T. Borgas. 2000. Airway mast cells and eosinophils correlate with clinical severity and airway hyperresponsiveness in corticosteroid-treated asthma. *J. Allergy Clin. Immunol.* 105: 752–759.
- Brightling, C. E., P. Bradding, F. A. Symon, S. T. Holgate, A. J. Wardlaw, and I. D. Pavord. 2002. Mast-cell infiltration of airway smooth muscle in asthma. *N. Engl. J. Med.* 346: 1699–1705.
- Asai, K., J. Kitaura, Y. Kawakami, N. Yamagata, M. Tsai, D. P. Carbone, F. T. Liu, S. J. Galli, and T. Kawakami. 2001. Regulation of mast cell survival by IgE. *Immunity* 14: 791–800.

16. Kalesnikoff, J., M. Huber, V. Lam, J. E. Damen, J. Zhang, R. P. Siraganian, and G. Krystal. 2001. Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. *Immunity* 14: 801–811.
17. Kawakami, T., and S. J. Galli. 2002. Regulation of mast-cell and basophil function and survival by IgE. *Nat. Rev. Immunol.* 2: 773–786.
18. Kawakami, T., and J. Kita. 2005. Mast cell survival and activation by IgE in the absence of antigen: a consideration of the biologic mechanisms and relevance. *J. Immunol.* 175: 4167–4173.
19. Gurish, M. F., P. J. Bryce, H. Tao, A. B. Kisselgof, E. M. Thornton, H. R. Miller, D. S. Friend, and H. C. Oettgen. 2004. IgE enhances parasite clearance and regulates mast cell responses in mice infected with *Trichinella spiralis*. *J. Immunol.* 172: 1139–1145.
20. Kita, J., J. Song, M. Tsai, K. Asai, M. Maeda-Yamamoto, A. Mocsai, Y. Kawakami, F. T. Liu, C. A. Lowell, B. G. Barisas, et al. 2003. Evidence that IgE molecules mediate a spectrum of effects on mast cell survival and activation via aggregation of the FcεRI. *Proc. Natl. Acad. Sci. USA* 100: 12911–12916.
21. Mehlhop, P. D., M. van de Rijn, A. B. Goldberg, J. P. Brewer, V. P. Kurup, T. R. Martin, and H. C. Oettgen. 1997. Allergen-induced bronchial hyperreactivity and eosinophilic inflammation occur in the absence of IgE in a mouse model of asthma. *Proc. Natl. Acad. Sci. USA* 94: 1344–1349.
22. Oettgen, H. C., T. R. Martin, A. Wynshaw-Boris, C. Deng, J. M. Drazen, and P. Leder. 1994. Active anaphylaxis in IgE-deficient mice. *Nature* 370: 367–370.
23. Bryce, P. J., M. L. Miller, I. Miyajima, M. Tsai, S. J. Galli, and H. C. Oettgen. 2004. Immune sensitization in the skin is enhanced by antigen-independent effects of IgE. *Immunity* 20: 381–392.
24. Abonia, J. P., J. Hallgren, T. Jones, T. Shi, Y. Xu, P. Koni, R. A. Flavell, J. A. Boyce, K. F. Austen, and M. F. Gurish. 2006. α-4 integrins and VCAM-1, but not MAdCAM-1, are essential for recruitment of mast cell progenitors to the inflamed lung. *Blood* 108: 1588–1594.
25. Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* 29: e45.
26. Gurish, M. F., H. Tao, J. P. Abonia, A. Arya, D. S. Friend, C. M. Parker, and K. F. Austen. 2001. Intestinal mast cell progenitors require CD49dβ7 (α4β7 integrin) for tissue-specific homing. *J. Exp. Med.* 194: 1243–1252.
27. Lantz, C. S., M. Yamaguchi, H. C. Oettgen, I. M. Katona, I. Miyajima, J. P. Kinet, and S. J. Galli. 1997. IgE regulates mouse basophil FcεRI expression in vivo. *J. Immunol.* 158: 2517–2521.
28. Yamaguchi, M., C. S. Lantz, H. C. Oettgen, I. M. Katona, T. Fleming, I. Miyajima, J. P. Kinet, and S. J. Galli. 1997. IgE enhances mouse mast cell FcεRI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. *J. Exp. Med.* 185: 663–672.
29. Arinobu, Y., H. Iwasaki, M. F. Gurish, S. Mizuno, H. Shigematsu, H. Ozawa, D. G. Tenen, K. F. Austen, and K. Akashi. 2005. Developmental checkpoints of the basophil/mast cell lineages in adult murine hematopoiesis. *Proc. Natl. Acad. Sci. USA* 102: 18105–18110.
30. Huang, C., A. Sali, and R. L. Stevens. 1998. Regulation and function of mast cell proteases in inflammation. *J. Clin. Immunol.* 18: 169–183.
31. Miller, H. R., and A. D. Pemberton. 2002. Tissue-specific expression of mast cell granule serine proteinases and their role in inflammation in the lung and gut. *Immunology* 105: 375–390.
32. Dillon, S. B., and T. T. MacDonald. 1986. Limit dilution analysis of mast cell precursor frequency in the gut epithelium of normal and *Trichinella spiralis* infected mice. *Parasite Immunol.* 8: 503–511.
33. Artis, D., N. E. Humphreys, C. S. Potten, N. Wagner, W. Muller, J. R. McDermott, R. K. Grencis, and K. J. Else. 2000. β₇ integrin-deficient mice: delayed leukocyte recruitment and attenuated protective immunity in the small intestine during enteric helminth infection. *Eur. J. Immunol.* 30: 1656–1664.
34. Pennock, J. L., and R. K. Grencis. 2004. In vivo exit of c-kit⁺/CD49d^{hi}/β₇⁺ mucosal mast cell precursors from the bone marrow following infection with the intestinal nematode *Trichinella spiralis*. *Blood* 103: 2655–2660.
35. Vermes, I., C. Haanen, H. Steffens-Nakken, and C. Reutelingsperger. 1995. A novel assay for apoptosis: flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled annexin V. *J. Immunol. Methods* 184: 39–51.
36. Bradding, P., A. F. Walls, and S. T. Holgate. 2006. The role of the mast cell in the pathophysiology of asthma. *J. Allergy Clin. Immunol.* 117: 1277–1284.
37. Marone, G., M. Triggiani, and A. de Paulis. 2005. Mast cells and basophils: friends as well as foes in bronchial asthma? *Trends Immunol.* 26: 25–31.
38. Galli, S. J., J. Kalesnikoff, M. A. Grimbaldston, A. M. Piliponsky, C. M. Williams, and M. Tsai. 2005. Mast cells as “tunable” effector and immunoregulatory cells: recent advances. *Annu. Rev. Immunol.* 23: 749–786.
39. Galli, S. J., S. Nakae, and M. Tsai. 2005. Mast cells in the development of adaptive immune responses. *Nat. Immunol.* 6: 135–142.
40. Korsgren, M., J. S. Erjefalt, O. Korsgren, F. Sundler, and C. G. Persson. 1997. Allergic eosinophil-rich inflammation develops in lungs and airways of B cell-deficient mice. *J. Exp. Med.* 185: 885–892.
41. Takeda, K., E. Hamelmann, A. Joetham, L. D. Shultz, G. L. Larsen, C. G. Irvin, and E. W. Gelfand. 1997. Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. *J. Exp. Med.* 186: 449–454.
42. Kobayashi, T., T. Miura, T. Haba, M. Sato, I. Serizawa, H. Nagai, and K. Ishizaka. 2000. An essential role of mast cells in the development of airway hyperresponsiveness in a murine asthma model. *J. Immunol.* 164: 3855–3861.
43. Ikeda, R. K., M. Miller, J. Nayar, L. Walker, J. Y. Cho, K. McElwain, S. McElwain, E. Raz, and D. H. Broide. 2003. Accumulation of peribronchial mast cells in a mouse model of ovalbumin allergen induced chronic airway inflammation: modulation by immunostimulatory DNA sequences. *J. Immunol.* 171: 4860–4867.
44. Yu, M., M. Tsai, S. Y. Tam, C. Jones, J. Zehnder, and S. J. Galli. 2006. Mast cells can promote the development of multiple features of chronic asthma in mice. *J. Clin. Invest.* 116: 1633–1641.
45. Mayr, S. I., R. I. Zuberi, M. Zhang, J. de Sousa-Hitzler, K. Ngo, Y. Kuwabara, L. Yu, W. P. Fung-Leung, and F. T. Liu. 2002. IgE-dependent mast cell activation potentiates airway responses in murine asthma models. *J. Immunol.* 169: 2061–2068.
46. Taube, C., X. Wei, C. H. Swasey, A. Joetham, S. Zarini, T. Lively, K. Takeda, J. Loader, N. Miyahara, T. Kodama, et al. 2004. Mast cells, FcεRI, and IL-13 are required for development of airway hyperresponsiveness after aerosolized allergen exposure in the absence of adjuvant. *J. Immunol.* 172: 6398–6406.
47. Williams, C. M., and S. J. Galli. 2000. Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. *J. Exp. Med.* 192: 455–462.
48. Mehlhop, P. D., M. van de Rijn, J. P. Brewer, A. B. Kisselgof, R. S. Geha, H. C. Oettgen, and T. R. Martin. 2000. CD40L, but not CD40, is required for allergen-induced bronchial hyperresponsiveness in mice. *Am. J. Respir. Cell Mol. Biol.* 23: 646–651.
49. Kohno, M., S. Yamasaki, V. L. Tybulewicz, and T. Saito. 2005. Rapid and large amount of autocrine IL-3 production is responsible for mast cell survival by IgE in the absence of antigen. *Blood* 105: 2059–2065.
50. Lantz, C. S., J. Boesiger, C. H. Song, N. Mach, T. Kobayashi, R. C. Mulligan, Y. Nawa, G. Dranoff, and S. J. Galli. 1998. Role for interleukin-3 in mast-cell and basophil development and in immunity to parasites. *Nature* 392: 90–93.
51. Beck, L. A., G. V. Marcotte, D. MacGlashan, A. Togias, and S. Saini. 2004. Omalizumab-induced reductions in mast cell FcεRI expression and function. *J. Allergy Clin. Immunol.* 114: 527–530.
52. Prussin, C., D. T. Griffith, K. M. Boesel, H. Lin, B. Foster, and T. B. Casale. 2003. Omalizumab treatment down-regulates dendritic cell FcεRI expression. *J. Allergy Clin. Immunol.* 112: 1147–1154.
53. Saini, S. S., A. D. Klion, S. M. Holland, R. G. Hamilton, B. S. Bochner, and D. W. MacGlashan, Jr. 2000. The relationship between serum IgE and surface levels of FcεR on human leukocytes in various diseases: correlation of expression with FcεRI on basophils but not on monocytes or eosinophils. *J. Allergy Clin. Immunol.* 106: 514–520.
54. Saini, S. S., D. W. MacGlashan, Jr., S. A. Sterbinsky, A. Togias, D. C. Adelman, L. M. Lichtenstein, and B. S. Bochner. 1999. Down-regulation of human basophil IgE and FcεRIα surface densities and mediator release by anti-IgE infusions is reversible in vitro and in vivo. *J. Immunol.* 162: 5624–5630.