

IL-13 Overexpression Predisposes to Anaphylaxis Following Antigen Sensitization¹

Padraic G. Fallon,* Claire L. Emson,[†] Philip Smith,* and Andrew N. J. McKenzie^{2†}

Anaphylaxis represents an extreme form of allergic reaction. This acute-phase component of allergy and asthma is triggered by allergen-induced degranulation of mast cells following the cross-linking of cell surface-bound, allergen-specific IgE, resulting in the liberation of inflammatory mediators and the development of bronchoconstriction. We used IL-13 transgenic mice to investigate the role of this Th2 cell-derived cytokine in the onset of allergic disease. Strikingly, IL-13-transgenic mice were highly predisposed to fatal anaphylaxis following Ag sensitization. This response correlated with substantially elevated levels of circulating Ag-specific IgE, mast cell degranulation, and histamine release. Furthermore, allergen exposure also induced phenotypic changes typical of asthma, including pulmonary fibrosis, goblet cell hyperplasia, elevated Th2 cytokines, eosinophilia, and airways occluded by mucus and Charcot-Leyden crystals. Expression of IL-4 was not required for the induction of IgE-mediated responses. These data represent the first characterization of a functional role for IL-13 in the generation of immediate hypersensitivity reactions and highlight the importance of IL-13 in the development of the symptoms of atopy. The systemic regulation of this response makes these mice an important resource for studying atopic responses. *The Journal of Immunology*, 2001, 166: 2712–2717.

Active anaphylaxis is the cause of fatalities following exposure to allergenic Ags in atopic individuals. Death results from the systemic release of inflammatory mediators following the cross-linking of receptor-bound IgE with Ag and the subsequent degranulation of mast cells, basophils, and eosinophils, inducing bronchoconstriction, shortness of breath, and lowered blood pressure (1, 2). These symptoms represent a classical immediate-type hypersensitivity reaction and are associated with the acute-phase component of allergy and asthma (3). In addition, late-phase allergic responses lead to airway remodeling characterized by epithelial hypertrophy, fibrosis, goblet cell hyperplasia, and airway occlusion due to mucus plugging and the deposition of Charcot-Leyden crystals by activated eosinophils (3). These reactions are co-ordinated by the release of a spectrum of immune-modulating cytokines, including IL-4, IL-5, IL-9, and IL-13, from mast cells and Th2 cells (1, 3). Genetic and immunological analyses of atopic individuals have revealed that Th2-type cytokines are causally associated with atopy (2, 4). Recently, elevated levels of IL-13 have been found to correlate with IgE and atopy in allergic asthma patients (5), and a search for genetic variants of the IL-13 gene and its signaling components has emphasized an important role for this cytokine in asthma and atopy (6). Experimental mouse models have also highlighted the importance of IL-13 in immune responses and atopy. Mice expressing IL-13 transgenes under the control of the IL-13 promoter and the lymphoid-specific CD2 locus control region have demonstrated that IL-13 can reg-

ulate IgE expression even in the absence of IL-4 expression. Furthermore, IL-13-deficient and IL-4R-deficient mice support an important *in vivo* role for IL-13, displaying impaired Th2 cell development (7, 8), inefficient responses to helminth infections (9, 10), and reduced basal IgE levels (7, 11). The role of IL-13 in experimental asthma models has also been examined. Blocking of IL-13 function using an IL-13 antagonist, or intratracheal administration of rIL-13, demonstrated that IL-13 plays a key role in the onset of airway hyperresponsiveness (AHR)³ (3) and the up-regulation of mucus-secreting goblet cells (12, 13). Furthermore, Zhu et al. (14) have reported that lung-specific expression of an IL-13 transgene induces an Ag-independent phenotype characterized by goblet cell hyperplasia, eosinophil infiltration, and increased AHR.

Here we describe the effect of IL-13 up-regulation on the development of immune responses in the context of Th2-inducing antigenic challenge. In experiments using transgenic mice expressing IL-13 in a lymphoid-specific manner (15), we find that exposure to Ag induces rapid and highly elevated production of Ag-specific IgE and that subsequent exposure to Ag induces fatal anaphylaxis with attendant mast cell degranulation and histamine release. In addition, this predisposition to immediate hypersensitivity is associated with a pulmonary pathology highly characteristic of asthma. Thus, IL-13 plays an important role in the systemic response to allergen challenge, in addition to regulating local tissue modification.

Materials and Methods

Mice and pulmonary model

IL-13-transgenic (IL-13Tg), IL-13Tg × IL-4^{-/-}, and wild-type littermates were prepared on a C57BL/6 background (15). Eight- to 10-wk-old female mice were used in this study. All mice were tagged s.c. with electronic chips, and to maintain objectivity, these were only scanned upon completion of the experiment. Synchronous pulmonary granulomas were induced by i.v. injection of mice with *Schistosoma mansoni* eggs. Mice were sensitized to schistosome eggs by i.p. injection of 5000 eggs. Two weeks later, sensitized and naive mice, four to six mice/group, were injected i.v. with

*Department of Pathology, University of Cambridge, Cambridge, United Kingdom; and the [†]Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom.

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² Address correspondence and reprint requests to Dr. Andrew McKenzie, Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K. E-mail address: anm@mrc-lmb.cam.ac.uk

³ Abbreviations used in this paper: AHR, airway hyperresponsiveness; IL-13Tg, IL-13 transgenic; RBL, rat basophilic leukemia cells; PAS, periodic acid Schiff.

5000 eggs. Serum and plasma were recovered and stored at -20°C . Data presented are from four separate experiments.

Immunological analysis

Serum levels of IL-4, IL-5, IL-13, and IFN- γ were determined by ELISA (11). Levels of histamine in plasma were assayed using a commercial histamine enzyme immunoassay kit (Immunotech, Marseille, France) according to the manufacturer's instructions. Total serum IgE was determined by ELISA using purified IgE as standard (BD PharMingen, San Diego, CA). Ag-specific IgE was detected using digoxigenin-labeled egg Ag (16).

Rat basophilic leukemia cells (RBL.2H3) were passively sensitized by incubation for 2 h at 37°C with a 1:20 dilution of mouse serum. To inactivate IgE, sera were heated at 56°C for 2 h. After gentle washing of the cells, egg Ag (25 ng/ml) was added for 1 h at 37°C . Degranulation of cells was determined by quantification of β -hexosaminidase activity in culture supernatants using the substrate *p*-nitrophenyl-*N*-acetyl- β -D-glucosamide (17). Total cellular enzymatic activity was measured in cell lysates (Triton X-100-treated). Because schistosome egg Ags elicit low levels (<5%) of spontaneous degranulation of RBL cells in the absence of sera, background Ag-induced degranulation was subtracted from serum + Ag-mediated degranulation. Data are expressed as the percentage of the mediator released relative to total cellular β -hexosaminidase activity. Data presented are mean and SD values from more than six mice per group and are representative of a minimum of three experiments performed.

Pulmonary histopathology

Following termination of mice, lungs were perfused via the right ventricle with 5 ml of formalin saline. Lungs were fixed in formalin saline, then paraffin-embedded, and 4- μm sections were cut. During preparation of histological sections, all slides were numerically coded and analyzed blind. Sections were stained with hematoxylin and eosin (cellular infiltration), toluidine blue (mast cells), Martius scarlet blue (collagen), periodic acid Schiff (PAS) (goblet cells), Giemsa (eosinophils), and Papanicolaou stain (eosinophilic crystals). Pulmonary fibrosis was quantified by differential staining of histological sections, with data expressed as micrograms of collagen per milligram of protein (18). The numbers of toluidine blue-stained mast cells were enumerated by counting the number of positive-stained cells per $\times 100$ field of view, with 20 fields counted per mouse. Mast cells were classified as either intact or degranulated, i.e., mast cells showing extrusion of >10% of cell granules. To quantify tissue eosinophilia, the numbers of peribronchial eosinophils in a high-field view ($\times 1000$ magnification) were counted; a minimum of 50 fields were counted per mouse. PAS-stained cells were counted using an arbitrary scoring system to quantify mucus secretion (12). PAS-stained cells in airway epithelia were measured using a numerical scoring system (0: <5% goblet cells; 1: 5–25%; 2: 25–50%; 3: 50–75%; 4: >75%). The sum of airway scores from each lung was divided by the number of airways examined, 20–50 airways per mouse, and expressed as mucus score in arbitrary units (AU). Autofluorescent eosinophilic crystals were examined on a Leica (Deerfield, IL) confocal microscope. Data are presented as mean and SD values from more than four mice per group, and are representative of three experiments performed.

Results

Fatal anaphylaxis in sensitized IL-13Tg mice

Allergic reactions are characterized by Th2 cytokine production and are believed to represent an inappropriate immune response that would normally be beneficial in the development of immunity to helminth parasite infection (9, 19, 20). Therefore, we used a pulmonary granuloma model in which egg Ags from the helminth parasite *S. mansoni* specifically produce a Th2 response in mice. This Th2 cytokine-mediated inflammation model evokes pulmonary changes that are characteristic of allergies/asthma, including induction of Th2 cytokines, elevated IgE, pulmonary eosinophilia, goblet cell hyperplasia, and airway remodeling (11, 16, 21). Unexpectedly, i.v. injection of eggs elicited immediate anaphylaxis in IL-13Tg mice that were presensitized to egg Ags; in four separate experiments, 0–75% of IL-13Tg mice died within 10 min of Ag challenge (Fig. 1). The symptoms observed in IL-13Tg mice after i.v. egg challenges were characteristic of fatal anaphylaxis in mice: bronchospasm, piloerection, tachypnea, and, ultimately, death. In contrast, comparably treated wild-type mice developed no symp-

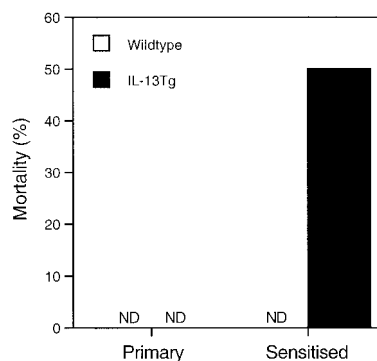


FIGURE 1. Fatal anaphylaxis of sensitized IL-13Tg mice (IL-13Tg) following injection of *S. mansoni* eggs. Injection of eggs i.v. into naive (primary) IL-13Tg or wild-type mice (primary or sensitized) did not cause mortality (ND, not detected). Data presented as mean mortality from four separate experiments with four to six mice/group. In all experiments performed to date, sensitized IL-13Tg mice were the only animals to display mortality.

toms (Fig. 1), and in all mouse strains tested with this model to date there have been no reports of fatal anaphylaxis. Pre-exposure to the Ag was essential for fatal anaphylaxis because egg challenge of naive transgenic mice did not cause symptoms (Fig. 1).

To determine the nature of this response, we assessed the levels of inflammatory mediators and IgE following Ag challenge. Significant elevation in plasma histamine levels was observed in the sensitized IL-13Tg mice as compared with wild-type controls (Fig. 2a). Furthermore, although the numbers of pulmonary mast cells in sensitized IL-13Tg mice (1.6 mast cells/10 fields of view) were only marginally increased relative to wild-type animals (1.4 mast cells/10 fields of view), the majority of mast cells (>95%) in transgenic mice that developed fatal anaphylaxis had undergone degranulation (Fig. 2b). Because Ag cross-linking of IgE bound to high-affinity receptors (Fc ϵ R1) on mast cells is one of the triggers

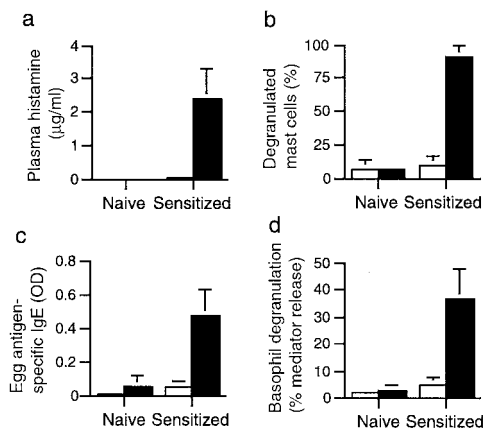


FIGURE 2. Elevated anaphylactic responses in sensitized IL-13Tg mice. Systemic histamine levels and pulmonary mast cell degranulation (a and b) and Ag-specific IgE and functional IgE (c and d) were assayed in naive and egg-sensitized wild-type and IL-13Tg mice. a, Plasma levels of histamine were substantially elevated in sensitized IL-13Tg animals. b, In lung sections, >95% of pulmonary mast cells had undergone degranulation in the sensitized transgenic mice. c, IL-13Tg-sensitized mice had elevated Ag-specific IgE. d, RBL cells passively sensitized with serum from immunized IL-13Tg mice displayed marked degranulation in the presence of egg Ags. Data are presented as mean and SD, more than six mice per group, and are representative of a minimum of three separate experiments. □, Wild type; ■, IL-13Tgs.

that elicits mast cell degranulation (1), we quantified serum levels of Ag-specific IgE in wild-type and IL-13Tg mice. We found that even within the short-term (14-day) challenge the IL-13Tg mice generated ~10-fold higher levels of egg Ag-specific IgE than wild-type control mice (Fig. 2c). Thus, increased expression of IL-13 enhances Ag-specific IgE as well as total serum IgE levels (15). We also tested whether serum from sensitized transgenic mice had functional IgE that could mediate degranulation of the RBL cell line in the presence of egg Ags *ex vivo*. Serum from sensitized IL-13Tg mice elicited substantial (40–50% of total cellular mediators released) degranulation of basophils in the presence of egg Ags (Fig. 2d), whereas serum from naive IL-13Tg mice or from naive or sensitized wild-type mice evoked limited degranulation of RBL cells (Fig. 2d). Heat inactivation of IgE by treatment of serum for 2 h at 56°C caused a reduction in the capacity of serum from sensitized transgenic mice to mediate RBL degranulation (data not shown), suggesting a dominant role for IgE in this process. Taken together, these data suggest that following exposure to egg Ags in the presence of elevated IL-13, there is a marked increase in the rate of production of Ag-specific IgE, which mediates Ag-specific degranulation of mast cells and the release of inflammatory mediators, resulting in the onset of anaphylaxis. Interestingly, serum levels of the Th2 cytokines IL-4, IL-5, and, as expected, IL-13 were substantially elevated in sensitized IL-13Tg mice compared with wild-type animals (Fig. 3), indicating that IL-13 production can induce higher levels of circulating Th2 cytokines, a phenotype that mirrors the cytokine deficit reported in IL-13-deficient mice (7).

Alterations in pulmonary architecture of sensitized IL-13Tg mice

Because the lungs represent the site where schistosome eggs lodge following *i.v.* challenge, and because pulmonary pathology is causally related to fatal anaphylaxis, we performed histological analysis on lungs from wild-type and transgenic mice. This analysis demonstrated profound perturbation of the pulmonary architecture of the IL-13Tg mice following Ag sensitization, resulting in fibrosis, epithelial hypertrophy, goblet cell hyperplasia, and eosinophil recruitment. Sensitized IL-13Tg mice displayed marked peribronchial fibrosis, with collagen deposition (blue stain) strikingly associated with airway epithelium throughout the pulmonary parenchyma, resulting in considerable thickening of the alveolar epithelium (Fig. 4a). In contrast, pulmonary collagen deposition in naive and sensitized wild-type mice as well as naive IL-13Tg animals was similar (Fig. 4a). Quantification of collagen deposition demonstrated a 3-fold elevation in pulmonary fibrosis in the sensitized IL-13Tg mice, whereas naive IL-13Tgs displayed only marginally elevated levels of collagen deposition compared with wild-type animals (Fig. 4b). The lungs of sensitized IL-13Tg mice also developed marked airway epithelial hypertrophy and goblet cell

hyperplasia within the bronchioles (Fig. 4, c and d), which were absent from sensitized wild-type mice (Fig. 4, c and d). The combined epithelial hypertrophy and mucus secretion caused occlusion of airways in sensitized IL-13Tg mice (Fig. 4, e and f). Within occluded airways were mucus, cellular infiltrate, and extracellular needle-like crystal structures (Fig. 4, e and f). These structures were confirmed to be eosinophilic crystals (the murine equivalent of Charcot-Leyden crystals in humans) by their fluorescence on Papanicolaou-stained sections (22) (Fig. 4g). The crystals were up to 150 μ m in length and formed lattices that contributed to the blockage of the airways. In mice, comparable eosinophilic crystals have been observed in a number of conditions where they are often associated with eosinophilia (23). Indeed, in contrast to the naive IL-13Tg and wild-type animals, sensitization of IL-13Tg mice evoked marked pulmonary eosinophilia associated with the hypertrophic bronchiolar epithelium (Fig. 4h). It is noteworthy that small numbers of eosinophilic crystals were also observed in the lungs of some naive IL-13Tg mice in the absence of overt eosinophilia.

Discussion

Our data highlight the importance of IL-13 in the generation and development of immediate hypersensitivity reactions following systemic Ag sensitization. They demonstrate that inappropriate elevation of IL-13 expression following Ag challenge predisposes to atopy, characterized by elevated Ag-specific IgE and Th2 cytokine responses, airway remodeling, goblet cell hyperplasia, and eosinophil infiltration with associated crystal deposition. Significantly, these morphological changes were accompanied by an increased susceptibility to fatal anaphylaxis induced by re-exposure to sensitizing Ag. Importantly, these effects are only mediated by antigenic challenge and constitute a systemic immune response culminating in the IgE-mediated anaphylaxis reaction, demonstrating that IL-13 may modify both the inductive phase of the allergic response to Ag and the terminal mediation of the response.

The cross-linking of IgE Fc ϵ R on mast cells, leading to mast cell degranulation, is probably the most important factor in generating the pathogenesis of atopic disorders. The inflammatory mediators released from the mast cell, including histamine, lead to the onset of symptoms such as bronchoconstriction, edema, and the recruitment of inflammatory cells. Although a number of studies have shown that IgE and mast cells are not essential for the generation of anaphylaxis, with both mast cell-deficient (24) and IgE-deficient mice developing anaphylaxis (25), it is clear that they play an extremely important role in the native immune reaction. We have shown that IL-13Tg mice produce highly elevated levels of Ag-specific IgE and that this can induce mast cell/basophil degranulation *in vivo* and *in vitro*. Although this pathway appears to represent the main route of immediate hypersensitivity displayed in the IL-13Tg mice, it is also possible that other cell types and IgG1 also play a role in this process.

The IgE-mediated allergic responses in Ag-sensitized IL-13Tg animals is associated with elevated serum levels of IL-13, but also increased expression of IL-4 and IL-5. Because IL-4 and IL-13 display biological overlap with respect to the induction of allergic responses (11), the elevated IL-4 production in IL-13Tg mice may represent an important contributing factor to the anaphylaxis in these animals. We have previously used IL-13Tg mice that do not express IL-4 (IL-13Tg $\times$ IL-4^{-/-}) to demonstrate that IL-13 can elicit IgE expression independently of IL-4 (15). Following schistosome egg sensitization, IL-13Tg $\times$ IL-4^{-/-} mice develop anaphylaxis responses at an order of magnitude comparable to that of IL-13Tg mice (mean $\pm$ SD of egg Ag-specific IgE 0.62 ± 0.2 OD in IL-13Tg $\times$ IL-4^{-/-} mice compared with 0.567 ± 0.1 OD in

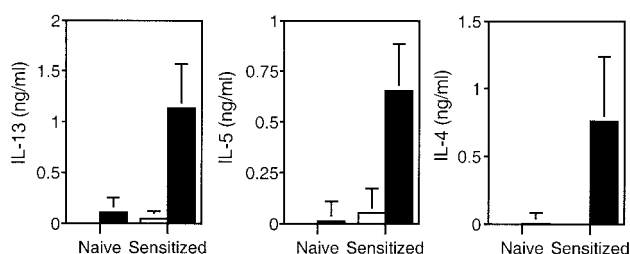
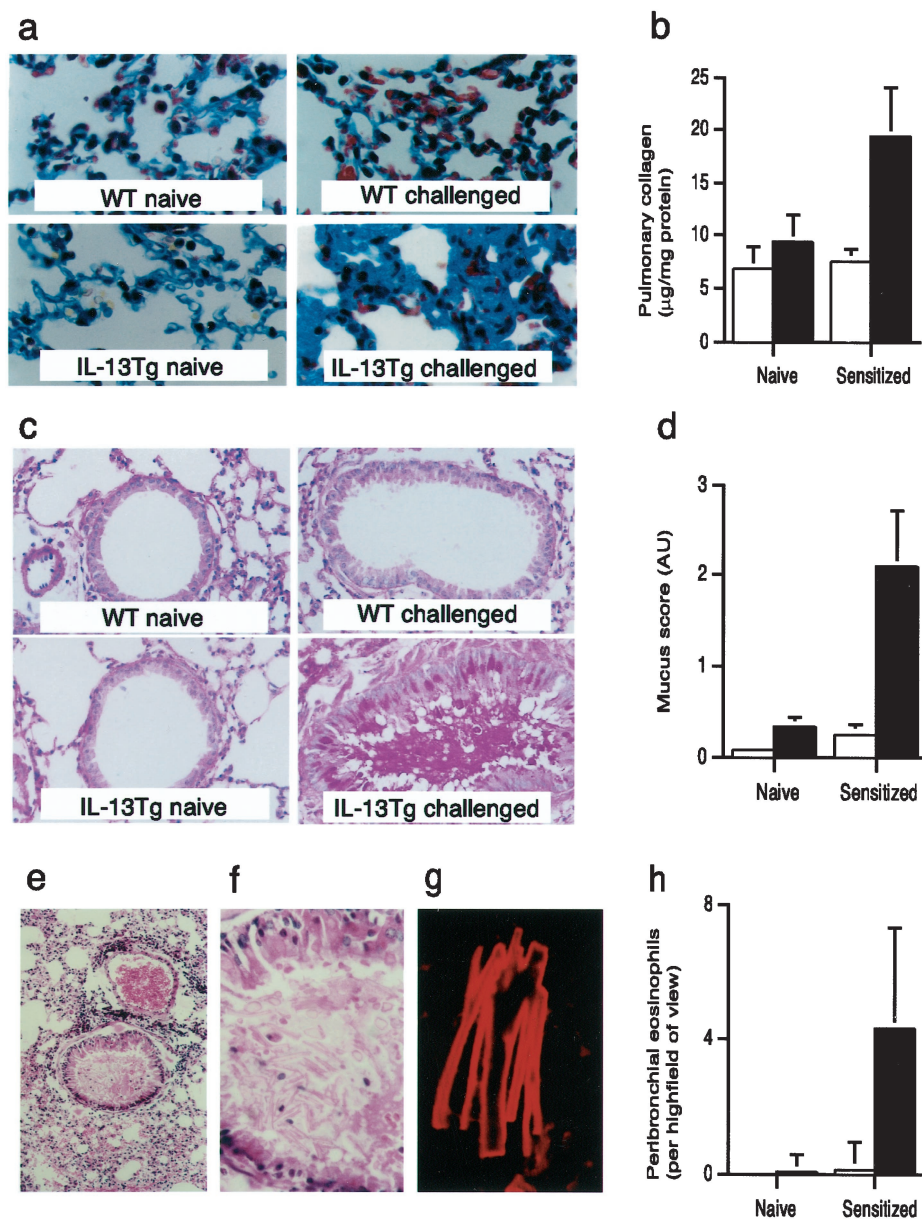


FIGURE 3. Elevated serum levels of Th2 cytokines in sensitized IL-13Tg mice. Serum cytokine levels are expressed as group mean and SD from six mice/group. Comparable data were obtained in three separate experiments. □, Wild type; ■, IL-13Tgs.

FIGURE 4. Alterations in the pulmonary architecture of IL-13Tg mice. *a*, Sensitized IL-13Tg mice had substantial deposition of collagen in the pulmonary parenchyma, causing thickening of alveolar epithelia (Martius scarlet blue stain; original magnification, $\times 400$). *b*, Quantification of pulmonary fibrosis illustrating up-regulation of collagen in transgenic mice after Ag sensitization. *c*, Sensitized IL-13Tg mice displayed partially occluded airways with marked epithelial hyperplasia and mucus cell metaplasia (PAS stain; original magnification, $\times 100$). *d*, Enumeration of PAS-stained goblet cells in airways was used to quantify epithelial mucus (expressed as mucus score) and confirmed the mucus cell metaplasia in sensitized transgenic mice. *e* and *f*, Within the blocked airways of sensitized IL-13Tg mice were large (up to 150 μm long) needle-like structures that formed lattice-like structures surrounded with mucus and cells (hematoxylin and eosin stain; original magnification, $\times 40$ and $\times 100$, respectively). *g*, Confocal microscopy of individual eosinophilic crystals (Papanicolaou stain). *h*, Elevated peribronchial eosinophilia in sensitized IL-13Tg mice, expressed as numbers of eosinophils per high-field view. $\square$, Wild type; $\blacksquare$, IL-13Tgs.



IL-13Tg mice). Serum from IL-13Tg or IL-13Tg $\times$ IL-4 $^{-/-}$ mice elicited 20–24% mediator release from RBL cells in the presence of schistosome egg Ags, whereas serum from sensitized wild-type mice elicited $<4\%$ basophil degranulation. In this experiment, no mortality was observed in any of the groups, and we are unable to comment on the importance of IL-4 in this process (data not shown). Our data further highlight that overexpression of IL-13 acts independently of IL-4 in the induction of certain allergic responses.

A number of studies on humans have identified a correlation between the expression of IL-13 and the development of atopy (26, 27). Furthermore, IL-13 expression and IL-13 gene polymorphisms have been found to associate with increased IgE expression in atopic patients (5, 28). A direct role for IL-13 in regulating lung epithelial cell function has also been reported, indicating that IL-13 may differentially modulate subsets of lung fibroblasts, inducing recruitment and activation of inflammatory cells (29). Only recently have experimental mouse models been used to investigate the potential roles of IL-13 in allergic responses. Such studies have highlighted the importance of IL-13 in the modulation of goblet

cell hyperplasia and as an uncharacterized effector molecule in AHR (12, 13). In addition, a recent study by Zhu et al. (14) demonstrated that overexpression of IL-13 using a lung tissue-specific promoter resulted in morphological changes similar to those observed in our study. However, these effects were not Ag-specific and failed to show the systemic alteration in circulating IgE, increased Th2 responses, or the onset of anaphylaxis, probably due to restricted expression of IL-13 to the lung tissue. In contrast, our study clearly shows that in a more physiological model, where IL-13 expression is regulated by the IL-13 promoter and a T cell enhancer and requires Ag stimulation, IL-13 is a potent modifier of IgE and fibrotic responses to allergen and plays a causal role in the generation of immediate hypersensitivity reactions.

Thus, IL-13 is an extremely important regulator of allergic responses, influencing a broad spectrum of Th2-driven effector functions and predisposing to atopy. Furthermore, these IL-13Tg mice represent an important model for studying the induction of such responses following allergen challenge and demonstrate that the therapeutic modulation of IL-13 may prove beneficial in regulating both tissue remodeling and systemic IgE responses to allergens.

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