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Marked Airway Eosinophilia Prevents Development of Airway Hyper-responsiveness During an Allergic Response in IL-5 Transgenic Mice

Takao Kobayashi, Koji Iijima, and Hirohito Kita

Tissue eosinophilia probably plays important roles in the pathophysiology of bronchial asthma and allergic disorders; however, this concept was challenged recently by controversial results in mouse models of bronchial asthma treated with anti-IL-5 Ab and the failure of anti-IL-5 therapy in humans. We have now used a unique model, IL-5 transgenic (TG) mice, to address a fundamental question: is airway eosinophilia beneficial or detrimental in the allergic response? After sensitization and challenge with OVA, IL-5 TG mice showed a marked airway eosinophilia. Surprisingly, these IL-5 TG mice showed lower airway reactivity to methacholine. Immunohistochemical analysis of the lungs revealed a marked peribronchial infiltration of eosinophils, but no eosinophil degranulation. In vitro, mouse eosinophils from peritoneal lavage fluids did not produce superoxide anion, but did produce an anti-inflammatory and fibrotic cytokine, TGF-β1. Indeed, the TGF-β1 levels in bronchoalveolar lavage fluid specimens from IL-5 TG mice directly correlated with airway eosinophilia (r = 0.755). Furthermore, anti-IL-5 treatment of IL-5 TG mice decreased both airway eosinophilia and TGF-β1 levels in bronchoalveolar lavage fluids and increased airway reactivity. Thus, in mice, marked eosinophilia prevents the development of airway hyper-reactivity during an allergic response. Overall, the roles of eosinophils in asthma and in animal models need to be addressed carefully for their potentially detrimental and beneficial effects. The Journal of Immunology, 2003, 170: 5756–5763.

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2 Address correspondence and reprint requests to Dr. Hirohito Kita, Departments of Medicine and Immunology, Mayo Clinic and Foundation, Rochester, MN 55905. E-mail address: kita.hirohito@mayo.edu

3 Abbreviations used in this paper: MBP, major basic protein; AHR, airway hyper-reactivity; BAL, bronchoalveolar lavage; BSL-1, biotinylated Griffonia (Banderaeae) simplicifolia lectin I; HSA, human serum albumin; PAF, platelet-activating factor; Penh, enhanced pause; TG, transgenic; LTC4, leukotriene C4.

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and human eosinophils. Second, the eosinophil in both mice and humans may be a “two-edged sword” or multifunctional; this would complicate the interpretations of IL-5 depletion or anti-IL-5 mAb. Indeed, in addition to their conventional effector functions, which are based on the release of lipid mediators and cationic granule proteins, eosinophils may functionally interact with immune cells or interstitial cells by presenting Ags (17) and producing cytokines (e.g., IL-4) and growth factors (e.g., TGF-β) (18). In particular, the association between human eosinophils and TGF-β1, a factor involved in immunoregulation and wound healing, has been studied extensively in patients with asthma (19, 20) and atop dermatitis (21).

In our research we have addressed a fundamental question: is airway eosinophilia beneficial or detrimental in the allergic response? Because previous studies that reduced eosinophilia by IL-5 gene knockout or by anti-IL-5 mAb treatment produced contradictory results, we used the opposite strategy. We used IL-5 transgenic (TG) mice and induced a vigorous accumulation of endogenous eosinophils into the airways during their allergic responses. Surprisingly, increased eosinophilia in the airways prevented allergen-induced AHR; this protective effect was probably due to the TGF-β produced by eosinophils.

Materials and Methods

Reagents

Human IL-5 (a gift from Schering-Plough Research Institute, Kenilworth, NJ), recombinant mouse IL-5 (R&D Systems, Minneapolis, MN), recombinant human eotaxin (R&D Systems), and recombinant murine eotaxin (PeproTech, Rocky Hill, NJ) were diluted in PBS containing 0.1% human serum albumin (HSA) at 100 μg/ml and stored at −70°C. Platelet-activating factor (PAF; Biomol, Plymouth Meeting, PA) was dissolved in absolute ethanol at 40 mM and stored at −20°C. PMA and calcium ionophore A23187 (Calbiochem, La Jolla, CA) were dissolved in DMSO at 5 mg/ml and 10 mM, respectively, and stored at −20°C. All agonists were diluted in reaction medium immediately before the experiments. Purified human serum IgG (Organon Teknika-Cappel, Malvern, PA) was stored at 4°C. Recombinant mouse IgG1, IgG2a, and IgG2b (ICN Pharmaceuticals, West Center, PA) were stored at −20°C. Biotinylated Grifonia (Banisteria) simplicifolia lectin I (BSL-I; Vector Laboratories, Burlingame, CA) was dissolved in PBS at 100 μg/ml and stored at −20°C. OVA, Percoll, HSA, and cytochrome c were obtained from Sigma-Aldrich (St. Louis, MO).

Mice

IL-5 TG mice were provided by Dr. K. Takatsu (Institute of Medical Science, Tokyo University, Tokyo, Japan) (22) and bred in Rochester, MN, under specific, pathogen-free conditions. Wild-type C3H/HeN mice (Charles River Laboratories, Wilmington, MA) were used as controls. All mice were males and were used at 7 wk of age.

OVA sensitization and challenge

The sensitization and challenge procedure with OVA was modified from the method described by Zhang et al. (23). Briefly, all mice were sensitized by an i.p. injection of 20 μg of OVA adsorbed to 1 mg of aluminum hydroxide gel (alum; Pierce, Rockford, IL) on days 0 and 14. Experimental mice were intranasally challenged with 50 μg of OVA in 50 μl of PBS under light tribromoethanol anesthesia on days 14, 25, 26, and 27. Control mice received intranasal PBS. To examine the role of IL-5, mice received 1 mg/kg of anti-IL-5 mAb (MAB405, TRFK5; R&D Systems) or control rat IgG (R&D Systems) in 100 μl of PBS i.p. on days 13 and 24. To neutralize the bioactivities of TGF-β, mice were intranasally instilled with 1 μg of anti-TGF-β1, -β2, and -β3 mAb (R&D Systems) or control mouse IgG1 (ICN Pharmaceutical) intranasally on days 25, 26, and 27.

Measurement of AHR

AHR was assessed on day 28 by methacholine-induced airflow obstruction in conscious mice in a whole body plethysmograph (Buxco Electronics, Troy, NY). Pulmonary airflow obstruction was measured by enhanced pause (Penh) as: Penh = (Te/RT − 1) × (PEF/PIF), where Penh is the enhanced pause (dimensionless), Te is the expiratory time, RT is the relaxation time, PEF is the peak expiratory flow (milliliters per second), and PIF is the peak inspiratory flow (milliliters per second) (24). Penh minute volume, tidal volume, and breathing frequency were obtained from chamber pressure, measured with a transducer connected to a pneumotachograph, and analyzed by system software (all from Buxco Electronics). To measure methacholine responsiveness, mice were exposed for 2 min to PBS, followed by incremental dosages (3–50 mg/ml) of aerosolized methacholine (freshly prepared in PBS); Penh was monitored for each dosage. Results were expressed for PBS and methacholine as the percentage of baseline Penh values before PBS exposure.

Collection of bronchoalveolar lavage (BAL) fluid

Immediately after measuring AHR, animals were injected i.p. with a lethal dose (250 mg/kg) of pentobarbital (Abbott Laboratories, Abbott Park, IL). The trachea was cannulated, the lungs were lavaged twice with 0.5 ml of HBSS, and the fluids were pooled. After centrifugation, the supernatant was collected and stored at −20°C. The cells were resuspended and counted using a hemocytometer. BAL cell differentials were determined with Wright-Giemsa stain; ≥200 cells were differentiated using conventional morphologic criteria. Concentrations of IL-5, IL-13, and TGF-β1 in the BAL fluid supernatants were measured by ELISA kits as directed by the manufacturer (R&D Systems). The sensitivities for IL-5, IL-13, and TGF-β1 assays were 4, 4, and 7 pg/ml, respectively.

Immunohistochemistry

After BAL fluid collection, the lung was fixed in 10% formalin and embedded in paraffin. A 6-μm section was stained with rabbit anti-mouse MBP as described previously (25).

Isolation of eosinophils

Human eosinophils were isolated from normal volunteers using a magnetic cell separation system (MACS; Miltenyi Biotech, Auburn, CA) as described previously (26). Mouse eosinophils were isolated as described previously (27) with minor modifications. Briefly, peritoneal lavage fluids were obtained using three lavage steps with PBS (total volume, 10 ml); these fluids were pooled and centrifuged. The pellet was resuspended in PIPES buffer with 1% bovine serum albumin (BSA) and incubated for 60 min on ice. After centrifugation the cells were resuspended in PIPES buffer with 1% DCS and streptavidin-conjugated magnetic beads (Miltenyi Biotech). After 15 min on ice, the eosinophils were separated by MACS and used immediately. Eosinophil purity determined by HEMA 3 stain (Biochemical Science, Swedesbord, NJ) was ≥95%.

Superoxide anion and leukotriene C4 (LT-C4) production

Eosinophil superoxide production was induced by various stimuli in poly-styrene 96-well flat-bottom tissue culture plates (Corning Costar, Cambridge, MA) and measured by reduction of cytochrome c, as described previously (22, 24). To immobilize Ig onto the wells, 50 μl of human IgG or mouse IgG1, IgG2a or IgG2b diluted in PBS at 100 μg/ml was added to the wells and incubated overnight at 4°C. After aspiration, the wells were blocked with 50 μl of 2.5% HSA in PBS for 2 h at 37°C. Freshly isolated eosinophils were washed and resuspended in HBSS with 0.03% gelatin, 10 mM HEPES and 200 mM cytochrome c at 5 × 106 cells/ml; 100 μl of cell suspension was dispensed into each well. The reactions were initiated with 100 μl of soluble stimuli, including IL-5, eosinin, PMA, or PAF. Wells coated with immobilized IgG received 100 μl of medium alone. Immediately after adding stimuli, absorbance at 550 nm was read in a microplate autoreader (Thermomax, Molecular Devices, Sunnyvale, CA), followed by repeated readings.

For LTC4 production, eosinophils were suspended in HBSS supplemented with 10 mM HEPES, 20 mM L-serine, and 5 mM glutathione at 4 × 106 cells/ml; 100 μl of cell suspension was dispensed into the wells, and the reactions were initiated with 100 μl of soluble stimuli or medium. After 1 h at 37°C, LTC4 levels in the supernatants were measured using an LTC4 ELISA kit as directed (Cayman Chemical, Ann Arbor, MI).

TGF-β1 production

Freshly isolated mouse eosinophils were washed and resuspended in RPMI 1640 (Celox Laboratories, St. Paul, MN) with 5% FBS (HyClone, Logan, UT) at 4 × 106 cells/ml; 100 μl of cell suspension was dispensed into the wells, and the reactions were initiated with 100 μl of soluble stimuli or medium. After incubation for 24 h at 37°C and 5% CO2, the supernatants were collected and stored at −20°C. Concentrations of TGF-β1 were measured by ELISA using a TGF-β1 kit as directed (R&D Systems).
Statistical analysis

Data are presented as the mean ± SEM for the numbers of mice or experiments indicated. The statistical significance of the differences between various treatment groups was assessed with Student’s t test.

Results

Allergen-induced airway eosinophilia in IL-5 TG mice

Previous studies used Abs to IL-5 or the IL-5 gene knockout model to deplete eosinophils in mice. We used a unique and opposite tactic and increased airway eosinophilia during the allergic response. The IL-5 TG mice that we used carried the mouse IL-5 gene ligated with a metallothionein promoter; these mice show massive eosinophilia in peripheral blood, bone marrow, and spleen, but without apparent organ involvement and dysfunction (22). As shown in Table I, OVA-sensitized and challenged wild-type C3H/HeN mice showed airway eosinophilia; OVA-sensitized, PBS-challenged wild-type mice did not. In contrast, OVA-sensitized and challenged IL-5 TG mice showed marked airway eosinophilia that was 158- and 69-fold higher than that in OVA-challenged wild-type mice and PBS-challenged IL-5 TG mice, respectively. Levels of IL-5 in BAL fluids were similar in PBS-challenged IL-5 TG mice (74 ± 21 pg/ml; n = 8) and OVA-challenged IL-5 TG mice (67 ± 12 pg/ml; n = 7); IL-5 levels were lower in OVA-challenged wild-type mice (19 ± 4 pg/ml; n = 8; p < 0.01) compared with OVA-challenged IL-5 TG mice. In contrast, IL-13 concentrations in BAL fluids were significantly increased in OVA-challenged IL-5 TG mice (140 ± 43 pg/ml; n = 7) compared with PBS-challenged IL-5 TG mice (15 ± 12 pg/ml; n = 8; p < 0.01). OVA-challenged wild-type mice (8 ± 3 pg/ml; n = 8) also showed a significant increase in IL-13 compared with PBS-challenged wild-type mice (<4 pg/ml; n = 8; p < 0.01).

Airway eosinophilia was examined histologically by staining the lung tissues with anti-MBP. As shown in Fig. 1A, eosinophils were rarely detected in alveoli or the peribronchial spaces in OVA-sensitized, PBS-challenged wild-type mice, but OVA-sensitized and challenged wild-type mice showed marked peribronchial infiltration of eosinophils (Fig. 1B). Lung tissues from OVA-sensitized, PBS-challenged IL-5 TG mice showed eosinophils localized mainly in the alveolar walls and rarely in the peribronchial space (Fig. 1C), suggesting that eosinophils are mainly in the small blood vessels feeding the alveoli. Lung tissues from OVA-sensitized and challenged IL-5 TG mice showed dramatically increased peribronchial eosinophilia (Fig. 1D). Furthermore, at higher magnification eosinophils were typically intact morphologically without apparent extracellular release of MBP protein or cell destruction (Fig. 1D, inset). Extracellular eosinophil granules containing MBP were seen occasionally; however, bronchial epithelium was not damaged.

Effects of airway eosinophilia on bronchial reactivity

We next examined the physiologic outcomes in this IL-5 TG mouse model. We used whole body plethysmography to examine airway reactivity in mice after methacholine inhalation. Previous studies suggested that human eosinophils are proinflammatory; thus, massive eosinophil infiltration should lead to increased bronchial reactivity. Fig. 2 shows that the OVA-sensitized and challenged wild-type mice exhibited significant AHR compared with OVA-sensitized, PBS-challenged wild-type mice (p < 0.05), consistent with other investigators (11, 12). The baseline airway reactivities of OVA-sensitized and PBS-challenged IL-5 TG mice and similarly sensitized wild-type mice were nearly identical. OVA-sensitized and challenged IL-5 TG mice showed increased airway reactivity compared with OVA-sensitized, PBS-challenged IL-5 TG mice (p < 0.05 at 6.3 mg/ml). Unexpectedly, OVA-sensitized and challenged IL-5 TG mice showed a significantly lower airway reactivity to methacholine than OVA-sensitized and challenged wild-type mice (p < 0.05 at 6.3 and 12.5 mg/ml). The methacholine dosages needed to increase airway reactivity to 250% of baseline were higher in IL-5 TG mice (~10.5 mg/ml) than in wild-type mice (~4.8 mg/ml). Thus, marked airway eosinophilia in IL-5 TG mice did not enhance, but, rather, inhibited, the development of AHR during an allergic response.

Proinflammatory functions of mouse eosinophils

To investigate the absence of any AHR despite massive airway eosinophilia in these mice, we characterized mouse eosinophils in vitro. In humans, many physiologic stimuli, such as cytokines (e.g., IL-5), lipid mediators (e.g., PAF), and immobilized Ig (e.g., IgG) (26, 28), some of which are strongly implicated in the allergic response, induce effector functions in eosinophils, such as LTC4 release and degranulation. Therefore, we used superoxide production to compare the proinflammatory functions of mouse and human eosinophils. Eosinophils were isolated from the peritoneal cavities of IL-5 TG mice and the peripheral blood of humans by negative selection. As shown in Fig. 3A, soluble agonists, such as PAF, IL-5, and eotaxin, and a pharmacologic agent, PMA, induced superoxide production from human eosinophils. Immobilized human IgG1 and several mouse IgG also activated human eosinophils, suggesting promiscuity of the FcγR response. In contrast, in Fig. 3B mouse eosinophils did not produce superoxide in response to any of these stimuli. Thus, mouse eosinophils did not respond to immobilized mouse IgG, but human eosinophils did. Furthermore, PMA stimulated both mouse and human eosinophils to produce comparable amounts of superoxide, suggesting that mouse eosinophils can produce superoxide. We suspected that the peritoneal eosinophils from IL-5 TG mice were altered or the cell isolation procedures affected cell functions. Alternatively, eosinophils from IL-5 TG mice might be intrinsically unresponsive to stimuli. Therefore, we repeated these experiments using eosinophils isolated from BAL fluids (94% purity) from OVA-sensitized and challenged IL-5 TG mice and eosinophils from peritoneal cavities of IL-5 TG mice without purification (80% purity). Furthermore, selected stimuli were also incubated with semipurified eosinophils.
(34% purity) from peritoneal cavities of OVA-sensitized and challenged wild-type BALB/c mice. However, for both experiments the results were virtually identical with those in Fig. 3B (data not shown).

We also examined LTC4 release from human and mouse eosinophils. The amounts of LTC4 production were as follows: 34 ± 15 pg/ml in human eosinophils with immobilized human IgG1, 205 ± 100 pg/ml in human eosinophils with 50 ng/ml human IL-5, <7.8 pg/ml in mouse eosinophils with immobilized mouse IgG2a, and <7.8 pg/ml in mouse eosinophils with 50 ng/ml mouse IL-5 (n = 3). Calcium ionophore A23187 (1 μM) induced vigorous LTC4 production by mouse eosinophils (9190 ± 2070 pg/ml). Overall, mouse eosinophils probably cannot produce proinflammatory mediators, such as superoxide anion and LTC4, when stimulated with physiologic agonists.

**Production of TGF-β1 by mouse eosinophils**

In human eosinophils, proinflammatory functions (e.g., superoxide production and degranulation) and cytokine production do not necessarily coincide (29), suggesting that different mechanisms exist for cytokine/growth factor production and inflammatory mediator release. TGF-β1 is important in asthma because of its functions in wound healing, tissue repair, and suppression of AHR (30, 31). Therefore, we examined whether mouse eosinophils produce TGF-β1. As shown in Fig. 4A, when isolated mouse eosinophils (IL-5 TG mice) were cultured in vitro, they spontaneously produced TGF-β1. Furthermore, TGF-β1 production by mouse eosinophils was enhanced by mouse IL-5 or mouse IL-3 by 52–73%, but not by mouse eotaxin up 500 ng/ml. Thus, mouse eosinophils do not make inflammatory mediators (i.e., superoxide, LTC4) in response to IL-5, but do make a growth factor, TGF-β1, in response to IL-5.

Increased levels of TGF-β1 were also detected in IL-5 TG mice in vivo. As shown in Fig. 4B, BAL fluids from OVA-sensitized and challenged IL-5 TG mice contained almost 10-fold higher TGF-β1 levels compared with BAL fluids from OVA-sensitized, PBS-challenged IL-5 TG mice. Furthermore, as shown in Fig. 4C, in these OVA-challenged IL-5 TG mice, the TGF-β1 levels positively correlated with the eosinophilia in BAL fluids. In BAL fluids there were no significant correlations between the levels of TGF-β1 and the numbers of macrophages, lymphocytes, and neutrophils (data not shown). A highly significant correlation between eosinophil numbers and TGF-β1 levels in BAL fluids was also seen in OVA-sensitized and challenged wild-type mice (r = 0.92; p < 0.0001; n = 12), suggesting that eosinophils from normal mice produce TGF-β1.
FIGURE 3. Superoxide production induced by immobilized IgGs, PAF, IL-5, or eotaxin in human (A) and IL-5 TG mouse (B) eosinophils. Human eosinophils were stimulated with immobilized IgGs (wells precoated with 100 μg/ml human (h) IgG1, mouse (m) IgG1, mlgG2a, or mlgG2b), 1 μM PAF, 5 ng/ml rhIL-5, 50 ng/ml human eotaxin, or 10 ng/ml PMA. Peritoneal lavage mouse eosinophils were stimulated similarly except mouse rhIL-5 and mouse eotaxin were used at the same concentrations. Superoxide production was measured by reduction of cytochrome c. Results are the mean ± SEM of three experiments.

In an earlier report lymphocytes engineered to produce TGF-β1 inhibited the development of AHR during Th2 cell-mediated airway inflammation (31). In our model, airway TGF-β, probably from eosinophils, profoundly affected airway physiology. When we completely blocked TGF-β by airway administration of neutralizing Ab in OVA-sensitized and challenged IL-5 TG mice, the baseline airway resistance before methacholine challenge was markedly increased (47 ± 15% increased Penh) compared with that with control Ig (13 ± 7% increased Penh; n = 4). Because of this elevated baseline, airway reactivity to methacholine could not be measured in these mice treated with anti-TGF-β.

Effects of anti-IL-5 on BAL eosinophilia, TGF-β1 levels, and airway reactivity

Thus, mouse eosinophils probably do not produce inflammatory mediators, but they do produce cytokines, such as TGF-β1. Furthermore, during an allergic response, this eosinophil-derived TGF-β1 may inhibit the development of AHR in IL-5 TG mice. The two previously identified cellular targets of IL-5 in mice are B cells (32) and eosinophils (33). In mice neither B cells nor Ab production are linked to decreased airway reactivity. Nonetheless, the attenuated development of AHR may arise from an unknown abnormality associated with IL-5 TG mice. Furthermore, cells other than eosinophils, such as lymphocytes, could produce TGF-β1 in mice. Therefore, we examined the effects of anti-IL-5 mAb (TRFK5) in OVA-sensitized and challenged IL-5 TG mice. Day 13 administration of anti-IL-5 to IL-5 TG mice reduced peripheral blood eosinophilia on day 21 by ~88% (41 ± 15 to 5 ± 2 × 10⁷/μl; n = 4). Anti-IL-5 did not affect the numbers of other cell types. As shown in Fig. 5, OVA-challenged, but not PBS-challenged, OVA-sensitized IL-5 TG mice showed increased BAL eosinophil numbers and increased TGF-β1 levels; anti-IL-5 mAb treated, OVA-sensitized and challenged mice showed decreased BAL eosinophil numbers (by 94%) and decreased TGF-β1 levels (by 57%) compared with mice treated with control Ig (p < 0.05).

FIGURE 4. In vitro and in vivo production of TGF-β1 in eosinophils from IL-5 TG mice. A, Peritoneal lavage mouse eosinophils were stimulated with mouse 5 ng/ml IL-5, 5 ng/ml IL-3, and 50 ng/ml eotaxin. After 24 h TGF-β1 production was measured by ELISA. Results are the mean ± SEM of three experiments. *, Significant difference (p < 0.05) from unstimulated cells (None). B and C, IL-5 TG mice were sensitized with OVA and challenged with PBS or OVA. Levels of TGF-β1 (B) and eosinophil numbers (C) were determined in BAL fluids. Results (B) are the mean ± SEM of three experiments, which showed similar results (total n = 11–13 in each group). The correlation (C) shows BAL fluid TGF-β1 levels and eosinophil numbers from three experiments with OVA-sensitized and challenged IL-5 TG mice.

FIGURE 5. Effects of anti-IL-5 Ab on BAL eosinophil numbers and TGF-β1 levels in BAL fluids. IL-5 TG mice were sensitized with OVA (days 0 and 14) and challenged with PBS or OVA (days 14 and 25–27). OVA-challenged mice were i.p. injected with anti-IL-5 or control Ab (days 13 and 24). Eosinophil numbers and TGF-β1 levels were determined in BAL fluids. Results are presented as the mean ± SEM of eight mice in each group. *, p < 0.05 between the OVA-challenged mice that received anti-IL-5 and control Ab.
treated mice challenged with OVA.

Flavonoids could down-regulate AHR during the allergic response, especially by inhibiting mast cell products (2, 3). However, by 1980, the discoveries of eosinophil cationic protein and eosinophil MBP (34) led to the hypothesis that eosinophils are proinflammatory cells involved in defense against large organisms, such as helmint parasites (4). Eosinophils can also make lipid mediators and produce oxygen metabolites (4, 5), and these proinflammatory roles of eosinophils may be involved in the pathophysiology of human asthma and allergic diseases. Indeed, many human clinical studies and animal models suggested that eosinophils are associated with or necessary for the AHR during the allergic airway response. In contrast, many mouse studies dissociated the AHR from eosinophil recruitment to the airways. These studies include the following: 1) airway eosinophilia can occur without AHR (35); and 2) AHR occurs without airway eosinophilia (12, 13, 36). AHR also occurred during an allergic response in the absence of murine MBP (37). Furthermore, several studies linked T cell mediators, such as IL-13 and IL-11, but not eosinophils, directly to allergen-induced AHR (15, 16, 38). These controversies now raise a critical question about the role of the eosinophil and tissue eosinophilia in bronchial asthma and allergic diseases: is it beneficial, detrimental, or just a bystander?

To address this issue in a unique manner, we used IL-5 TG mice to increase eosinophilia during the allergic response. In contrast, previous models had reduced eosinophilia by mAb or gene knockout (9–14). Another rationale for using IL-5 TG mice was the duration of airway eosinophilia. The airways of patients with untreated bronchial asthma are continuously exposed to IL-5 and eosinophils; due to chronic airway inflammation and occasional exacerbations, this exposure may persist for years. In contrast, most mouse models of bronchial asthma and allergic responses temporally provoke airway eosinophilia by allergen challenge; thus, airway physiology is studied during acute eosinophilic inflammation. Therefore, IL-5 TG mice, showing both chronic airway eosinophilia and allergen-induced acute eosinophilia, could better reflect the protracted course of human asthma and allergic diseases. Using this model we found no difference in baseline airway reactivity between IL-5 TG mice and wild-type animals, suggesting that eosinophilia per se does not lead to AHR. After OVA challenge the increased airway eosinophilia in IL-5 TG mice did not increase AHR, but, surprisingly, inhibited allergen-induced AHR. Perhaps AHR is induced by a factor(s) produced by T cells, such as IL-13, during the allergen challenge (15, 16, 38) and is counteracted by eosinophils and their products. Indeed, OVA-challenged mice showed increased IL-13 levels in BAL in this study.

Thus, our results seemingly contradict those from a previous study by Lee et al. (10), who demonstrated that overproduction of IL-5 in the lung epithelium led to marked airway eosinophilia and AHR. However, a careful comparison of both studies suggests logical and potential reasons for these differences. In the study by Lee et al. (10) the promoter for the Clara cell secretory protein CC10 was used, resulting in a dramatic increase in airway IL-5 compared with our study (269,000 ± 37,000 pg/lavage (10) vs 74 ± 21 pg/ml (our study)). Their markedly increased IL-5 levels may have contributed to both the peribronchial eosinophilia and the striking structural changes in the airways, including the expansion of bronchus-associated lymphoid tissue, epithelial hypertrophy, and focal collagen deposition (10). These pathologic changes probably resulted in AHR without Ag sensitization and challenge (10). Therefore, this previous study was not able to study the effects of airway eosinophilia on the development of AHR induced by allergen challenge and perhaps by a factor(s) produced by T cells, such as IL-13.

Human eosinophils potentially produce >20 cytokines, chemokines, and growth factors (18). Eosinophil-derived cytokines and growth factors may profoundly influence the allergic inflammatory response, but there is no in vivo evidence to directly support such a hypothesis. Although several factors produced by mouse eosinophils could down-regulate AHR during the allergic response, we suspect that TGF-β1 is the most likely. TGF-β1 is strongly associated with eosinophil numbers, and anti-IL-5 markedly influenced eosinophil numbers, TGF-β1 levels, and airway reactivity. Furthermore, complete blockade of TGF-β in OVA-challenged IL-5 TG mice induced profound airway resistance without methacholine challenge; indeed, the resistance precluded any methacholine challenge.

The association between human eosinophils and TGF-β1 has been studied extensively. In human asthma, activated eosinophils are a major source of TGF-β1 by in situ hybridization (19) and immunohistochemistry (20). Isolated human eosinophils produce both TGF-β1 protein (39) and TGF-β1 bioactivity (40). Eosinophil-derived TGF-β1 activity may not be limited to inhibiting AHR, but it may extend to immunoregulation, wound healing, tissue repair, and fibrosis. In mice, eosinophils have been implicated

![FIGURE 6.](http://www.jimmunol.org/Download) Effects of anti-IL-5 on methacholine-induced AHR in IL-5 TG mice. Mice were sensitized with OVA and challenged with PBS or OVA; OVA-challenged mice were injected i.p. with anti-IL-5 or control Ig (same schedule as in Fig. 5). Airway reactivity in response to 50 mg/ml methacholine, compared with baseline values before methacholine (as 100%), was measured using whole body plethysmography. Results are the mean ± SEM of two experiments, with a total of eight mice in each group. *, p < 0.05 between the mice challenged with PBS and the anti-IL-5-treated mice challenged with OVA.
in bleomycin-induced pulmonary fibrosis (41) and ectopic splenic bone formation (42). In human atopic skin, eosinophil-derivered TGF-β1 was also temporally associated with myofibroblast formation and with repair and remodeling events after an allergic response (21). Thus, the roles of eosinophils and eosinophil-derived TGF-β1 may be more diverse and profound than previously recognized.

We did not detect released eosinophil granule proteins in bronchial mucosa of OVA-sensitized and challenged wild-type and IL-5 TG mice (Fig 1); this is consistent with similar in vivo models (37, 43). An extensive morphological study of eosinophils during allergic inflammation in mice did not show any signs of eosinophil degranulation (44). It is possible that the allergen challenge models in mice, and perhaps those in humans, may lack a critical tissue environmental factor(s) involved in the vigorous degranulation of eosinophils; this factor would be common in real human diseases. However, our parallel and quantitative analyses of mouse and human eosinophil functions emphasize the inability of mouse eosinophils to produce proinflammatory mediators in response to several physiologic agonists (Fig 3). Indeed, in humans, inhalation of rIL-5 in patients with asthma increased airway eosinophilia, sputum eosinophil cationic protein levels, and AHR (45), suggesting that IL-5 alone is sufficient to activate human eosinophils and to induce physiological changes in the airways. On the other hand, like human eosinophils (39, 40), mouse eosinophils produced TGF-β1 and retained their ability to up-regulate TGF-β1 production in response to IL-5 and IL-3 (Fig 4A). Furthermore, mouse eosinophils also made proinflammatory mediators in response to pharmacologic agents such as PMA (Fig 3) and calcium ionophore A23187. Although the mechanisms involved in these functional differences between mouse and human eosinophils are beyond the scope of this study, we can speculate. Human eosinophils release proinflammatory mediators and produce cytokines/growth factors, but these functions are sometimes dissociated. For example, stimulation of human eosinophil FcγRII by immobilized ligands induces degranulation and superoxide production, but inhibits GM-CSF production (29). Conversely, soluble ligands for FcγRII induce GM-CSF production, but they do not induce degranulation or superoxide production (29). Therefore, some intra-cellular or membrane component(s) that enables eosinophils to react with immobilized ligands or large surfaces may be missing in mouse eosinophils. Perhaps selective evolutionary pressures, such as defense against certain parasites, on murine vs human eosinophils during the past 75–100 million years have contributed to species differences in eosinophil-mediated activities (46). Mouse eosinophils lack protective functions against helminths important in human infections, such as Schistosoma mansoni (47).

How is our study relevant to the human allergic response? The inability of murine eosinophils to produce proinflammatory mediators in response to stimuli involved in allergic inflammation needs consideration. In addition, these IL-5 TG mice and their marked airway eosinophilia may overestimate the protective aspects of eosinophils. Nonetheless, in the allergen challenge in human airways, the late phase airway narrowing occurs before the peak of eosinophil infiltration, and eosinophils persist after the resolution of airway narrowing (48, 49). During a cutaneous allergen challenge in atopic individuals, after the peak of the late phase response, eosinophils are present in the skin (21). Thus, eosinophils and tissue eosinophils may play a role(s) in resolving inflammation and airway reactivity and in the subsequent airway remodeling during and after the allergic response. Furthermore, eosinophils are present in the gastrointestinal tracts of normal individuals (50) and untreated healthy mice (51), suggesting a homeostatic role for eosinophils in mucosal tissues. Therefore, in addition to their pro-inflammatory effects, the protective effects of eosinophils may need to be considered when interpreting clinical studies in patients with asthma and allergic diseases, especially in allergen challenge studies. Furthermore, it will be important to clarify the circumstances under which eosinophils are critical sources of proinflammatory mediators and of protective factors and to delineate the signals that regulate their production and secretion. The idea that eosinophils might dampen the allergic response needs to be revisited with modern technologies.

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References
