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The immunoregulatory functions of IL-4 and IL-5 have identified these cytokines as primary targets for the resolution of airways inflammation and bronchial hyperreactivity in asthma. However, the individual contribution of each of these cytokines and of IL-5-regulated eosinophilia to the induction of airways hyperreactivity in mouse models of asthma remains highly controversial. In this investigation, we have used IL-4- and IL-5-deficient mice of the same genetic background in combination with inhibitory mAbs to these cytokines to identify unequivocally the contribution of these factors to the induction of airways hyperreactivity. Sensitization and aeroallergen challenge of wild-type mice with OVA induced pathological changes to the respiratory epithelium, airways eosinophilia, and hyperreactivity to β-methacholine. Inhibition of the actions of IL-4 and/or IL-5 did not abolish airways hyperreactivity, and in the case of IL-4-deficient mice pretreated with anti-IL-5 mAb, airways hyperreactivity persisted in the absence of pronounced airways inflammation. Airways hyperreactivity was abolished only by anti-CD4+ mAb treatment. However, aeroallergen challenge of IL-5-/- mice showed that morphologic changes to the airways were critically linked to IL-5 and eosinophilia. This investigation demonstrates the existence in BALB/c mice of a novel CD4+ T cell pathway for modulating airways hyperreactivity. These findings may provide an explanation for the dissociation of airways eosinophilia from the development of airways hyperreactivity observed in some cases of asthma and in animal models of this disease. The Journal of Immunology, 1998, 161: 1501–1509.

The underlying cellular and molecular mechanisms predisposing to the development of enhanced bronchial reactivity to spasmogenic stimuli (airways hyperreactivity) in asthmatics are unknown. Characterization of the immunopathogenesis of asthma suggests that allergen-specific CD4+ T cells of the Th2 phenotype play a central role in initiating and sustaining an asthmatic response by regulating the production of IgE and the effector function of mast cells and eosinophils (1–7). Infiltration of the airways by CD4+ Th2 type cells and eosinophils is a predominant feature of late phase asthmatic responses, and the presence of these cells and their inflammatory products in the lung often correlates with disease severity and the degree of airways hyperreactivity (8–14). Furthermore, the Th2 cytokines IL-4 and IL-5 have also been implicated in the etiology of asthma and play pivotal roles in orchestrating the inflammatory response in animal models (1, 3–18) of this disease. IL-4 is thought to be a critical factor for the regulation of T cell commitment to the CD4+ Th2 phenotype and is known to regulate IgE production by B cells (19, 20). In contrast, IL-5 regulates eosinophil development, activation, and recruitment of this leukocyte to sites of allergic inflammation (21–25). Furthermore, both cytokines have been implicated in regulating the development of airways hyperreactivity in response to allergen inhalation in animal models of asthma (25–27).

The immunomodulatory functions of IL-4 and IL-5 have identified these cytokines as key therapeutic targets for the relief of airways inflammation and obstruction in asthma. There is, however, opposing evidence as to the comparative significance of each cytokine in the mechanisms underlying the induction of airways hyperreactivity (25–35). In particular, airways eosinophilia, which is regulated by IL-5, is not always implicated in the development of airways hyperreactivity in asthma or in animal models of this disease (15, 27–30, 32–34, 35). Recently, mouse models of allergic airways inflammation have been used to provide insights into the potential contribution of individual inflammatory cells and molecules to the pathogenesis of asthma. In IL-5-deficient (IL-5-/-) mice of the C57BL/6 strain, we have demonstrated an obligatory role for this cytokine in the induction of eosinophilia, in morphologic changes to the airways, and in the development of airways hyperreactivity to β-methacholine in response to aeroallergen challenge (25). IL-5 and associated eosinophilia were also shown to regulate the induction of airways hyperreactivity during allergic inflammation in IL-4-/- mice (129Sv × C57BL/6 background) (26). Our investigations support the hypothesis that increased levels of eosinophils and their products in the lung underlie pathologic changes to the airways and enhanced bronchial reactivity in asthma (5, 8–13). In marked contrast, an essential role for IL-4 and not IL-5 or eosinophils in the development of aeroallergen-induced airways hyperreactivity was observed by Corry et al. in BALB/c mice treated with inhibitory mAbs to these cytokines (27). Significantly, airways hyperreactivity was reduced but not abolished by

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3 Abbreviations used in this paper: IL-5-/-, interleukin-5 deficient; IL-4-/-, IL-4 deficient; BALF, bronchoalveolar lavage fluid; PVDF, polyvinylidene difluoride; MBP, major basic protein.
anti-IL-4 mAb treatment (27). Furthermore, antagonism of leukotriene function in BALB/c mice inhibited the accumulation of mucus and eosinophils in the airways but did not attenuate airways hyperreactivity in response to aeroallergen challenge (34). Similar investigations in BALB/c mice and other strains have also suggested that IgE in association with airways eosinophilia is required for the induction of enhanced airways reactivity to spasmogens (28, 31, 35).

The innate contributions of IL-4, IL-5, and the effector pathways of eosinophils and mast cells to the induction of airways hyperreactivity in various strains of mice, in conjunction with different sensitization protocols, have been postulated to explain the apparent discordant observations described above (36). C57BL/6 mice are genetically deficient in some mast cell-derived inflammatory mediators, which could make this strain resistant to mast cell-mediated airways hyperreactivity (37). In contrast to C57BL/6 mice, BALB/c mice produce high levels of IL-4 and IgE in response to sensitization (27). Thus, airways hyperreactivity has been proposed to be predominantly regulated by IgE-mediated activation of mast cells in BALB/c mice and solely regulated by an IL-5-eosinophil-dependent mechanism in C57BL/6 mice (36). However, this hypothesis does not explain why eosinophils recruited to the airways in response to allergen inhalation in BALB/c mice do not become activated and thus contribute through the release of inflammatory mediators to the induction of airways hyperreactivity. An alternative hypothesis is that the contribution of eosinophils to the development of airways hyperreactivity in BALB/c mice may be masked by the coexistence of a pathway(s) that operates independently of this leukocyte.

The aim of this investigation was to ascertain whether multiple physiologic pathways predispose to aeroallergen-induced airways hyperreactivity in BALB/c mice. In this investigation, we have employed IL-4−/− and IL-5−/− mice of the BALB/c strain, in combination with inhibitory mAbs for these cytokines, in an attempt to unequivocally dissect the contribution of these factors and eosinophils to the development of airways hyperreactivity during allergic inflammation. Subcutaneous (27) and intraperitoneal (25) sensitization protocols were also compared to determine whether the route of Ag administration affects the contribution of inflammatory cells and cytokines to pathophysiologic features of the inflammatory response. Aeroallergen-induced airways hyperreactivity was shown to be present in IL-5−/− or IL-4−/− mice even when these mice were additionally treated with anti-IL-4 or anti-IL-5 mAbs. However, IL-5 and airways eosinophilia were essential for the development of morphologic changes to the airways. Thus, airways hyperreactivity persisted in the absence of pronounced airways inflammation and gross structural changes to the respiratory epithelium, but was ablated by anti-CD4+ mAb. Our data indicate that CD4+ T cells operate at least two pathways that can act independently to induce airways hyperreactivity in BALB/c mice. The coexistence of parallel pathways may account for the dissociation of airways eosinophilia from the development of airways hyperreactivity in some cases of asthma and in models of this disease in BALB/c mice and may have important implications for the treatment of asthma.

Materials and Methods

Induction of allergic airways inflammation

BALB/c IL-4−/− mice (provided by Dr. N. Noben-Trauth, National Institutes of Health (38)) or IL-5−/− mice (BALB/c 8th-10th generation derived from IL-5−/− C57BL/6 (24), 0 to 10 wk of age, were sensitized by i.p. injection with 50 μg OVA/1 mg Alhydrogel (Commonwealth Serum Laboratories (CSL), Parkville, Australia) in 0.9% sterile saline on days 0 and 12. Nonsensitized mice received 1 mg of Alhydrogel in 0.9% saline. On day 24, the appropriate groups of mice were aeroallergen challenged with OVA (nonsensitized mice received saline only) as previously described (25, 26). Twenty-four hours after the last aeroallergen challenge, airways hyperreactivity was measured, or mice were sacrificed by cervical dislocation and their airways characterized for inflammatory infiltrates and morphologic changes. Lung responses of IL-4−/− mice or IL-5−/− mice were compared with wild-type mice, which were simultaneously treated. In some experiments, wild-type mice received 1 mg Alhydrogel in 0.9% saline on day 0 and 12 and were then aeroallergen challenged with OVA. To determine whether the dose, route, and frequency of allergen challenge affected the contribution of IL-5 and eosinophils to the induction of allergic airways disease, mice were also s.c. injected with a lower dose of OVA (25 μg/1 mg Alhydrogel) weekly for 4 wk and subsequently aeroallergen challenged (three times daily at 5-day intervals) as described by Corry et al. (these investigators had previously reported no role for eosinophils in the development of airways hyperreactivity by using this model (27)). Mice were treated according to Australian National University Animal Welfare guidelines and were housed in a specific pathogen-free facility.

Pretreatment of mice with anti-IL-4, anti-IL-5, or anti-CD4 mAbs

IL-5−/− mice were injected i.p. with anti-IL-4 mAb (1 mg of 11B11) 24 h before i.p. injections with OVA and then weekly throughout the experimental protocol. IL-4−/− mice were injected i.p. with anti-IL-5 mAb (2 mg, TRFK-5) on days 23 and 27. Anti-CD4 mAb (0.75 mg of GK1.5) was administered before aeroallergen challenge on day 23 and on days 26 and 29. Mice also received the appropriate concentration of isotype control Ab (BGL113). All Abs were administered in sterile saline.

Characterization of lung morphology and leukocytes in blood, tissue, and bronchoalveolar lavage fluid (BALF)

Lung tissue representing the central (bronchi-bronchiole) and peripheral (alveoli) airways were fixed in 10% phosphate-buffered formalin, sectioned, and stained with May-Grunwald-Giemsa solution or hematoxylin-eosin. Leukocytes in the blood, BALF, and lung were identified by morphologic criteria and quantitated as previously described (25).

Measurement of airways hyperreactivity

Airways hyperreactivity was measured with a bronchospasm transducer (Ugo Basil 7020), which was coupled to a Lab Mac/8 analysis station (AdInstruments, Sydney, Australia) as previously described (25, 26). Changes in respiratory overflow volume were determined during cumulative i.v. administration of β-methacholine. The increase in respiratory overflow volumeprovoked by β-methacholine is represented as a percentage of the maximal overflow volume obtained by totally occluding the tracheal cannula. The concentration that induced a maximal response in OVA-sensitized and aerosolized wild-type mice is shown for all other groups.

Preparation of whole lung homogenates

Homogenates were prepared after cannulating the trachea and perfusing the airways with a 1-ml solution of 1% collagenase/PBS at 4°C. The lungs were then removed, resuspended in HBSS/10% FCS, and vigorously vortexed before being incubated at 37°C for 30 min. The resulting cell suspension was homogenized and filtered through nylon mesh (70 μM) and centrifuged at 500 × g for 5 min at 4°C. The resulting pellet was then resuspended in MLC/10% FCS, filtered through FACS gauze, and centrifuged at 500 × g for 5 min at 4°C. Cells were then resuspended in MLC/10% FCS and used for OVA or anti-CD3 stimulation assays.

IL-5 production by T cells in cultures of whole lung homogenates after stimulation with OVA or anti-CD3

Lung homogenates were cultured (2 × 106 cells/well) in MLC/10% FCS on OVA (50 μg/well) or anti-CD3 (5 μg/ml)-coated U-bottom 96-well plates (100 μl/well) for 72 h (26). Cell-free culture supernatants were then collected and stored in aliquots at −70°C until IL-5 levels were determined.

Measurement of IL-5 levels in culture supernatants

IL-5 concentrations were determined in the supernatants from OVA (50 μg/ml)- or anti-CD3 (5 μg/ml)-stimulated lung cell homogenates (26). Briefly, 96-well round-bottom immunosassay plates were coated with rat anti-mouse IL-5 mAb (5 μg/ml, clone TRFK-5). Plates were blocked with 5% FCS/PBS for 1 h and incubated with serial dilutions of cultured supernatants or standard murine IL-5 at 37°C. Murine IL-5 was detected with
biontylated rat anti-mouse IL-5 (3 μg/ml; TRFK-4). Plates were incubated with streptavidin-conjugated peroxidase (Jackson Immunoresearch Laboratories, West Grove, PA) for 1 h at 37°C, washed, and ABTS substrate solution (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added. After an appropriate incubation time, the reaction was stopped with 0.1 M SDS solution. Plates were read in a microplate reader (Bio-Tek Instruments, Winslow, VT) at 480 nm with reference to 405 nm. The sensitivity of the ELISA system was 0.5 ng/ml of IL-5.

Detection of major basic protein in the BALF

BALF was centrifuged (350 × g 4°C) for 5 min. Aliquots of the supernatant were removed and stored at −70°C until determined by immunoblot analysis. Dot blots were performed at room temperature using polyvinylidene difluoride (PVDF) membranes (Schleicher and Schuell, Dassel, Germany) that had been presoaked in 100% methanol for 1 min and then incubated in TBS solution (20 mM Tris-HCl, pH 7.5/150 mM NaCl) for 2 min. BALF samples were serially diluted (initial dilution, 1:2) in TBS solution and applied to the PVDF membranes. PVDF membranes were then air dried and washed with 100% methanol for 1 min and immersed in TBS solution for 4 min. Membranes were then blocked with 1% BSA/0.05% Tween solution (1% biotin-qualified BSA/20 mM Tris-HCl, pH 7.5/150 mM NaCl/0.05% Tween 20) for 20 min, washed with TBS solution, and incubated with rabbit anti-mouse-major basic protein (MBP) Ab in TBST solution (1:2000) for 1 h. Membranes were then washed with TBST solution for 5 min and incubated with alkaline phosphatase-conjugated goat anti-rabbit Ig (Sigma, St. Louis, MO) for 45 min. Finally, membranes were washed with TBST solution and incubated with Western blue-stabilized substrate (Promega, Madison, WI). Reactions were stopped with deionized water when bands of interest had reached the desired intensity.

Determination of OVA-specific Ab titers by ELISA

OVA-specific Abs were detected in the sera from OVA-treated mice as previously described (26).

Results

Characterization of allergic airways inflammation in IL-5−/− mice

Aeroallergen challenge of OVA-sensitized wild-type mice resulted in an increase in lymphocyte, neutrophil, macrophage, and eosinophil numbers in the BALF (Fig. 1A) in comparison with mice treated with saline and aerosolized with saline or OVA (Fig. 1A). In saline-immunized mice, no significant differences in inflammatory cell numbers were observed between saline- and OVA-aerosolized treatments (results for individual cell populations × 10^3/ml of BALF for saline-immunized and saline- or OVA-aerosolized treatment, respectively, are: lymphocytes, 0.84 ± 0.10 vs 0.97 ± 0.13; eosinophils not detected; neutrophils, 0.35 ± 0.09 vs 0.51 ± 0.10; macrophages, 1.84 ± 0.5 vs 3.5 ± 0.7; n = 5). Furthermore, there was no significant change in airways responsiveness to β-methacholine in these two groups. Percentage of maximal airways occlusion for saline-immunized and saline- or OVA-aerosolized treatment was 20.5 ± 2.4 vs 7.0 ± 2.8 (n = 5). Data are shown for a dose of 75 μg/kg β-methacholine, which induces a maximal response in wild-type mice. The composition of individual leukocyte populations in the BALF were not significantly different between mice that had been sensitized i.p. or s.c.

(A). Cell numbers in saline-treated IL-5−/− mice were similar to those observed in saline-treated wild-type mice (A). The number of macrophages in the BALF of saline-treated IL-5−/− mice was not significantly different than in the OVA-treated IL-5−/− groups (i.p. or s.c.). Neutrophils and eosinophils were not detected in the BALF of saline-treated IL-5−/− mice. Lymphocyte numbers were <0.5 cells/ml BALF × 10⁴ in saline-treated IL-5−/− mice. No significant differences were observed between inflammatory cell numbers in s.c. or i.p. OVA-treated IL-5−/− mice. C. Only numbers of eosinophil and lymphocyte numbers were significantly decreased in sensitized IL-4−/− mice (p < 0.05) compared with WT mice (A).

FIGURE 1. Characterization of inflammatory cells in wild-type (WT), IL-4−/−, and IL-5−/− mice. Inflammatory cell numbers in WT mice that were injected i.p. or s.c. with saline (Sal) or OVA (A), IL-5−/− mice that were injected i.p. or s.c. with OVA (B), and IL-4−/− mice that were sensitized by i.p. injection with OVA (C). Leukocytes were identified by morphologic criteria and qualified as described previously (25). All data were obtained at day 31. Data represent the mean ± SEM for groups of five to six mice. Corresponding WT data for groups B and C are shown in A. The significance of differences between experimental groups was analyzed using the unpaired Student’s t test. Differences were considered significant if p < 0.05 (A), *p < 0.05, **p < 0.005, and ***p < 0.001 compared with saline treatment. No significant differences were observed between s.c. and i.p. groups treated with OVA. B, All cell populations were significantly (p <0.005) reduced in sensitized IL-5−/− mice compared with wild-type mice.
Table I. Detection of MBP in BALF from nonsensitized or sensitized (OVA) and aeroallergen-challenged WT, IL-4−/− mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Presence of MBP</th>
<th>Morphologic changes to airways</th>
<th>IL-4−/− (i.p. or s.c.)</th>
<th>IL-5−/− (i.p. or s.c.)</th>
<th>IL-4−/− + Isotype Control Ab</th>
<th>IL-4−/− + Anti-IL-5 mAb</th>
<th>Eosinophil Supernatants</th>
<th>T Cell Supernatants</th>
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</thead>
<tbody>
<tr>
<td>Saline</td>
<td>No</td>
<td>No</td>
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<tr>
<td>WT</td>
<td>No</td>
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<tr>
<td>IL-4−/−</td>
<td>No</td>
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<tr>
<td>IL-5−/−</td>
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<tr>
<td>IL-4−/− + Isotype Control Ab</td>
<td>No</td>
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<tr>
<td>IL-4−/− + Anti-IL-5 mAb</td>
<td>No</td>
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<tr>
<td>Eosinophil Supernatants</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>No</td>
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<tr>
<td>T Cell Supernatants</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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</table>

*Cell-free BALF samples were assayed for the presence of MBP by immunoblot analysis as described in Material and Methods. All data were obtained on day 31. MBP was detected as a strong positive signal (+) or at background levels (−). Results represent an analysis of five samples per experimental group.
1C). Notably, eosinophil numbers in the BALF of OVA-treated IL-4−/− mice (3.15 × 10^4 ± 0.87, n = 6) (Fig. 1C) were significantly elevated in comparison with OVA-treated IL-5−/− mice (0.0625 × 10^4 ± 0.02, n = 6) (Fig. 1B), and MBP was detected at similar intensities in BALF from sensitized and aeroallergen-challenged wild-type and IL-4−/− mice (Table I). Furthermore, marked alterations in the morphologic integrity of the airways occurred in sensitized IL-4−/− mice (Fig. 4). IL-4−/− mice could also mount a pronounced blood and peribronchial eosinophilia that was not significantly different between aeroallergen-challenged wild-type and IL-4−/− mice (results not shown), as previously described in 129Sv × C57BL/6 mice deficient in this factor (26). Moreover, aeroallergen-induced morphologic changes to the airways (Fig. 4D) and airways hyperreactivity (Fig. 2) were not attenuated in IL-4−/− mice and were observed in the absence of OVA-specific IgE and in the presence of reduced levels of IgG1 (Fig. 3, A and B). These results indicate that in BALB/c mice, IL-4 is not obligatorily involved in the generation of aeroallergen-induced airways hyperreactivity or lung damage.

FIGURE 3. OVA-specific Igs in OVA-sensitized and aeroallergen-challenged wild-type (WT), IL-5−/−, and IL-4−/− mice. Serum OVA-specific IgE (A) and IgG1 (B) levels in OVA-sensitized and aeroallergen-challenged WT, IL-5−/−, and IL-4−/− mice. Serum titers for OVA-specific Igs were determined by ELISA on day 31 (see Materials and Methods). No OVA-specific Igs were detected in saline-challenged mice. Data represent the mean ± SEM for groups of five to six mice. The significance of differences between experimental groups was analyzed using the unpaired Student’s t test. Differences were considered significant if p < 0.05; A, *p < 0.05 compared with WT; B, *p < 0.05 compared with WT.

FIGURE 4. Histologic analysis of lung sections from wild-type (WT), IL-4−/−, and IL-5−/− mice. A, WT mice that were not sensitized and were exposed to an aerosol of saline; B, WT mice exposed to OVA; C, IL-5−/− mice exposed to OVA; and D, IL-4−/− mice exposed to OVA. Leukocytes were identified by morphologic criteria. Sections shown are representative of 10 sections of lung per mouse from three mice in each groups. Lung tissue was stained with May-Grunwald-Giemsa solution. Sections A–D, ×420
T cell production of IL-5 in IL-4<sup>−/−</sup> mice

In other mouse strains, we have demonstrated that airways eosinophilia and hyperreactivity can be regulated by pathways that operate independently of IL-4 but that are intimately associated with IL-5 production by CD4<sup>+</sup> T cells (26). In the present investigation, stimulation of T cells with anti-CD3 or OVA in homogenates of lungs taken from aeroallergen-challenged IL-4<sup>−/−</sup> BALB/c mice resulted in significant production of IL-5 (albeit less than that observed with preparations from wild-type mice; results not shown) but failed to further attenuate aeroallergen-induced airways hyperreactivity in BALB/c mice. Mice were sensitized (i.p.) and aerosolized. Pretreatment of IL-5<sup>−/−</sup> mice with anti-IL-5 mAb during periods of both systemic sensitization and aerosolization did not affect eosinophilia or airways responses to β-methacholine (Fig. 4A). This finding is consistent with the notion that there is another pathway regulating aeroallergen-induced airways hyperreactivity in BALB/c mice that is not dependent on IL-4 or IL-5 and that this pathway likely contributes to the airways responses to β-methacholine (Fig. 6). To determine the role of IL-5 and eosinophils in the induction of airways hyperreactivity and lung damage that occurred independently of IL-4, anti-IL-5 mAb was administered to IL-4<sup>−/−</sup> mice pretreated with inhibitory mAbs to IL-5, IL-4, CD4, or isotype control Ab. Responses in IL-4<sup>−/−</sup> mice pretreated with anti-IL-5 mAb, anti-IL-4 mAb, or isotype control Ab. Responses in IL-4<sup>−/−</sup> mice pretreated with anti-IL-5 mAb, βGL113, or anti-CD4 mAb; and B, measurement of airways hyperreactivity to β-methacholine in nonsensitized and sensitized aeroallergen-challenged IL-4<sup>−/−</sup> and IL-5<sup>−/−</sup> mice pretreated with inhibitory mAbs to IL-5, IL-4, CD4, or isotype control. Leukocytes were identified by morphologic criteria and quantified as previously described (25). Airways constriction was measured by determining changes in respiratory overflow volume during cumulative i.v. administration of β-methacholine. Data are represented as a percentage of the maximal overflow volume (100%) obtained by totally occluding the tracheal cannula. Data are shown for a dose of 75 μg/kg, which induced a maximal response in IL-4<sup>−/−</sup> mice pretreated with βGL113 (these mice gave equivalent response to WT mice (Fig. 2)). The data represent the mean ± SEM for five to six groups of mice. Data were obtained on day 31. The significance of differences between experimental groups was analyzed using the unpaired Student’s t test. Differences were considered significant if p < 0.05. A, *p < 0.05, **p < 0.005, or ***p < 0.001 compared with IL-4<sup>−/−</sup> mice pretreated with βGL113 Ab. B, ***p < 0.001 compared with IL-4<sup>−/−</sup> mice pretreated with βGL113 Ab. Responses in IL-4<sup>−/−</sup> mice were not significantly different from those in WT mice (Fig. 2).

IL-4<sup>−/−</sup> mice with anti-IL-5 mAb and anti-CD4 mAb (or anti-CD4 mAb alone; result not shown) significantly reduced lymphocyte numbers (but not neutrophil or macrophage numbers; result not shown) in the BALF and abolished airways hyperreactivity (Fig. 6, A and B). The histologic sections of the lungs of these mice resembled those of saline-treated mice, but in some regions adjacent to the airways, foci of lymphocyte clusters were observed. Collectively, these data indicate that there is another pathway regulating aeroallergen-induced airways hyperreactivity in BALB/c mice that is not dependent on IL-4 or IL-5 and that this pathway...
Discussion

Infiltration of eosinophils into the airways has been both associated with and disassociated from the development of airways hyperreactivity in asthma and a number of animal models of this disease (15, 25–35). In this investigation, by using IL-5−/− and IL-4−/− BALB/c strain in combination with inhibitory mAbs for these cytokines, we have identified two pathways that are critically regulated by CD4+ T cells operating independently and in parallel in the development of allergic airways disease. IL-5-regulated eosinophilia was critical for the induction of aeroallergen-induced lung damage in BALB/c mice, and this pathway operates in other mouse strains that have been examined (25, 26). However, a second T cell-regulated pathway, which predominately regulates airways hyperreactivity and was not associated with pronounced morphologic changes to the airways, was also observed. This pathway appears to operate primarily in BALB/c mice, as we have shown that inhibition of the actions of IL-5 or in combination with IL-4 abolishes the development of enhanced airways reactivity to spasmogens in other strains (25, 26). Thus, results in IL-5−/− BALB/c mice support observations that in this strain airways hyperreactivity can develop independently of IL-5 and eosinophilia (27, 34). Recently, antagonism of leukotriene formation in the lungs of BALB/c mice has also been shown to dissociate airways hyperreactivity from the development of allergen-induced mucus plugging and eosinophilic infiltration of the airways (34). However, eosinophil-induced pathologic changes to the allergic lung have been shown to contribute directly to the development of airways hyperreactivity in other strains of mice (25, 26) (also CBA IL-5−/− mice, unpublished observation). Thus, it is likely that the contribution of the IL-5/eosinophil pathway is masked in the BALB/c strain, although an effect was detectable with i.p. sensitization model (unpublished observation). Furthermore, OVA or anti-CD3 stimulation of T cells obtained from lungs of both strains of IL-4−/− mice after aeroallergen challenge also resulted in the production of significant quantities of IL-5, and in these mice Ag-induced eosinophilia and pathologic changes to the airways were abolished by treatment with anti-IL-5 mAb. However, unlike IL-4−/− BALB/c mice with anti-IL-5 mAb during primary sensitization (27). Furthermore, OVA or anti-CD3 stimulation of T cells obtained from BALB/c mice, are similar to those obtained with IL-4−/− 129Sv × C57BL/6 mice (26). A significant eosinophilia and airways hyperreactivity was also observed by Corry et al. in wild-type BALB/c mice after treatment with anti-IL-4 mAbs during primary sensitization (15). Additionally, treatment of BALB/c mice with anti-IL-4 mAbs during systemic sensitization did not further inhibit aeroallergen-induced airways hyperreactivity. Collectively, these investigations indicate that multiple T cell-regulated mechanisms underlie the development of airways hyperreactivity in BALB/c mice. It will be interesting to determine whether the CD4+ T cell mechanism modulating airways hyperreactivity in this strain is directly induced by novel CD4+ T cell-derived factors and/or downstream inflammatory processes.

While a number of cells and molecules contribute to the mechanism underlying the regulation of airways hyperreactivity in mice, only CD4+ T cells have been shown exclusively to regulate disease pathogenesis (48–50). Recently, the transfer of enriched naive T cell populations from a strain of mice that displayed inherent hyperreactivity to methacholine to a hyporeactive strain conferred airways hyperreactivity to this spasmogen in the absence of Ag challenge (51). The increased airways reactivity to methacholine was associated with CD4+ T cells and occurred in the absence of eosinophilia, pronounced inflammation, and morphologic changes to the airways. While the mechanism of T cell activation in the absence of Ag is obscure, these investigations support our conclusions that factors secreted from CD4+ T cells play fundamental roles in determining the level of airways reactivity to cholinergic stimuli. Moreover, there is increasing evidence that CD4+ T cell driven inflammation of the airways is a primary factor...
for the induction of the inflammatory cascade and bronchial hyperreactivity in asthma (1, 4–6, 10–13).

In this investigation, we have shown that a pathway intimately regulated by CD4+ T cells acts independently of IL-4 and IL-5 to modulate airways hyperreactivity in BALB/c mice. IL-5-regulated eosinophilia was essential for the induction of Ag-induced morbidity in BALB/c mice. IL-5-regulated T-lymphocytes, mast cells, neutrophils and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. J. Allergy Clin. Immunol. 92:397.


