Interleukin 5 Deficiency Abolishes Eosinophilia, Airways Hyperreactivity, and Lung Damage in a Mouse Asthma Model

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Summary

Airways inflammation is thought to play a central role in the pathogenesis of asthma. However, the precise role that individual inflammatory cells and mediators play in the development of airways hyperreactivity and the morphological changes of the lung during allergic pulmonary inflammation is unknown. In this investigation we have used a mouse model of allergic pulmonary inflammation and interleukin (IL) 5–deficient mice to establish the essential role of this cytokine and eosinophils in the initiation of aeroallergen-induced lung damage and the development of airways hyperreactivity. Sensitization and aerosol challenge of mice with ovalbumin results in airways eosinophilia and extensive lung damage analogous to that seen in asthma. Aeroallergen-challenged mice also display airways hyperreactivity to β-methacholine. In IL-5–deficient mice, the eosinophilia, lung damage, and airways hyperreactivity normally resulting from aeroallergen challenge were abolished. Reconstitution of IL-5 production with recombinant vaccinia viruses engineered to express this factor completely restored aeroallergen-induced eosinophilia and airways dysfunction. These results indicate that IL-5 and eosinophils are central mediators in the pathogenesis of allergic lung disease.

Characterization of the cellular and chemical events of asthma suggests that inflammatory cells play a central role in the clinical expression and pathogenesis of the disease (1). Clinical investigations show a correlation between the presence of activated inflammatory cells, histological changes to pulmonary tissue, and the development of airways hyperreactivity (2–5). The cellular composition of the inflammatory infiltrate in the lung is characterized by increased numbers of activated neutrophils, mast cells, eosinophils, and monocytes, and the recruitment and/or activation of these cells appears to be controlled by the secretion of cytokines and chemotactic agents from antigen-stimulated T lymphocytes (4–8). After accumulation at sites of allergic inflammation, these inflammatory cells release mediators (e.g., platelet-activating factor, leukotrienes, prostaglandins, histamine, cytokines, chemokines, and cationic proteins) that have the potential to augment the inflammatory response and induce tissue damage and dysfunction (1).

In asthma, airways limitation may be induced by IgE-dependent release of spasmogenic mediators from activated mast cells (9, 10). Similarly, eosinophils and neutrophils, by releasing granular proteins, lipid mediators, and oxygen metabolites, also have the potential to induce pulmonary damage and airways hyperreactivity and to exacerbate the inflammatory response (10–13). Whereas these cells and the mediators they release may contribute to a complex inflammatory cascade, their individual roles in the events that initiate the morphological and functional changes of the asthmatic lung are unknown.

Recently, there has been increasing interest in the involvement of eosinophils in the pathogenesis of asthma (14). In the majority of clinical studies, pulmonary eosinophilia has been recognized as a predominant feature of the inflammatory infiltrate, which often correlates with disease severity (2–5, 14). A number of inflammatory mediators (platelet-activating factor, leukotriene B_4, IL-5, and chemokines [RANTES, macrophage chemoattractant protein, macrophage inflammatory protein, and eotaxin]) have chemotactic activity for eosinophils and are candidates for the control of eosinophilia in asthma (15–18). Of these mediators, only IL-5 regulates the growth, differentiation, and activation of eosinophils (19–21).

Furthermore, activated $CD4^+$ Th2-type cells, which can produce IL-5 and several other cytokines, are activated in a greater proportion in asthmatic individuals (6–8, 13). Elevated levels of IL-5 and cells expressing mRNA for IL-5 are also found in the blood and lung secretions from asthmatic patients or after allergen-induced late-phase asthmatic responses and correlate with the degree of eosino-
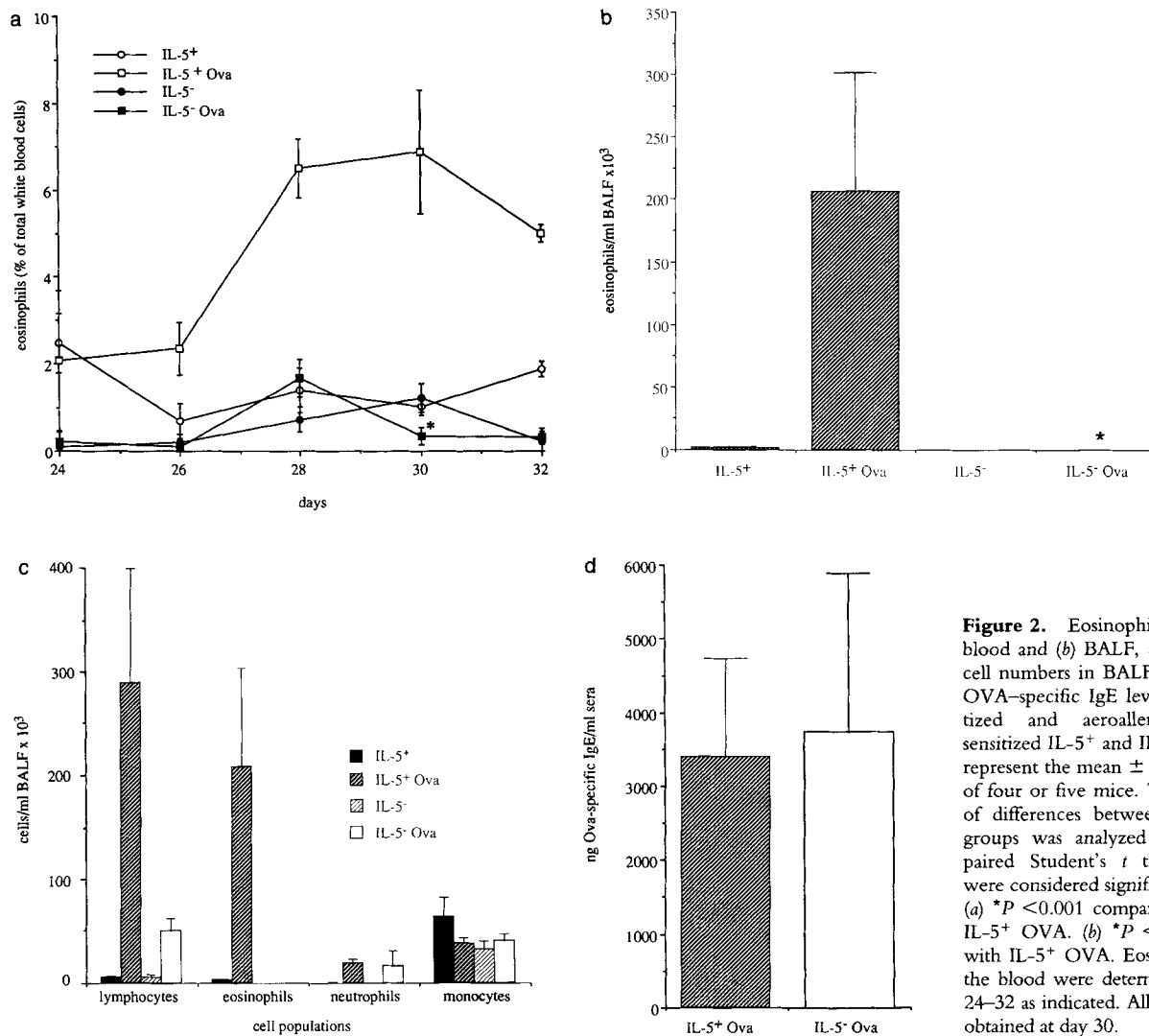
philic inflammation (6–8, 22). Thus, in the limited number of investigations to date, IL-5 production by activated T lymphocytes and pulmonary eosinophilia have been associated with asthma. However, a number of other cytokines are also produced by activated T lymphocytes, and eosinophils are only one of a number of inflammatory cell types regularly found in the pulmonary infiltrates in asthma. In animal studies, mAbs directed against IL-5 have been shown to suppress, but not abolish, pulmonary eosinophilia in response to antigen challenge, and the correlation of this effect with changes in lung morphology or airways hyperreactivity remains controversial (23, 24). Furthermore, evidence for the role of eosinophils in the development of airways hyperreactivity is conflicting (3, 4, 23, 25, 26).

In this work we have tried to gain evidence of the rela-

tive importance of IL-5 and eosinophils in the pathogenesis of asthma by using a mouse asthma model and IL-5–deficient (IL-5⁻) mice (26a). We have established a mouse model of allergic pulmonary inflammation that mimics allergen-induced late-phase asthmatic responses. In this model, aeroallergen challenge of sensitized mice results in airways hyperreactivity to β-methacholine (this airways spasmogen is used widely as a clinical tool to assess disease severity in asthmatic patients), severe pulmonary eosinophilic inflammation, and changes in lung morphology that closely parallel those observed in the asthmatic lung (1, 3). We have used the mouse model of allergic inflammation and IL-5⁻ mice (which completely lack this cytokine) as a novel way of gaining unequivocal data concerning the role of IL-5 in pulmonary eosinophilia and the development of
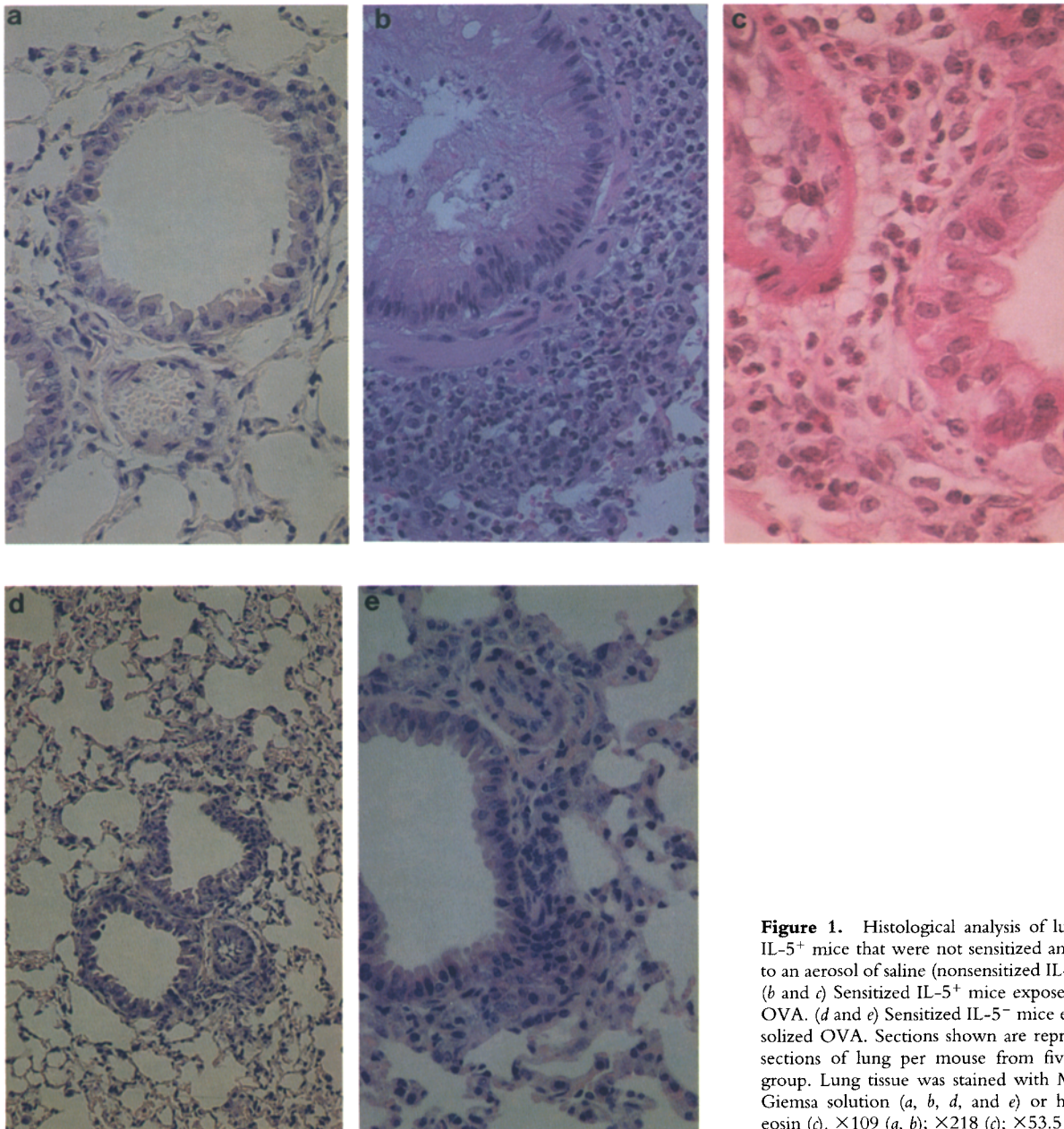
Figure 1. Histological analysis of lung sections. (a) IL-5⁺ mice that were not sensitized and were exposed to an aerosol of saline (nonsensitized IL-5⁻ not shown). (b and c) Sensitized IL-5⁺ mice exposed to aerosolized OVA. (d and e) Sensitized IL-5⁻ mice exposed to aerosolized OVA. Sections shown are representative of 10 sections of lung per mouse from five mice in each group. Lung tissue was stained with May–Grunwald–Giemsa solution (a, b, d, and e) or hematoxylin and eosin (c). ×109 (a, b); ×218 (c); ×53.5 (d); ×107 (e).

a

10

eosinophils (% of total white blood cells)

8

6

4

2

0

—o— IL-5+
—□— IL-5 + Ova
—•— IL-5-
—■— IL-5- Ova

*

24   26   28   30   32

days

b

350

300

250

200

150

100

50

0

eosinophils/ml BALF x10³

*

IL-5+   IL-5+ Ova   IL-5-   IL-5- Ova

c

400

300

200

100

0

cells/ml BALF x 10³

■ IL-5+
▨ IL-5+ Ova
▨ IL-5-
□ IL-5- Ova

lymphocytes   eosinophils   neutrophils   monocytes

cell populations

d

6000

5000

4000

3000

2000

1000

0

ng Ova-specific IgE/ml sera

IL-5+ Ova   IL-5- Ova

Figure 2. Eosinophil numbers in (a) blood and (b) BALF, (c) inflammatory cell numbers in BALF, and (d) serum OVA–specific IgE levels in nonsensitized and aeroallergen-challenged, sensitized IL-5+ and IL-5- mice. Data represent the mean ± SEM for groups of four or five 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) *$P < 0.001$ compared with day 30 IL-5+ OVA. (b) *$P < 0.01$ compared with IL-5+ OVA. Eosinophil levels in the blood were determined from days 24–32 as indicated. All other data were obtained at day 30.

aeroallergen-induced lung damage and airways hyperreactivity in vivo. In IL-5- mice, the airways hyperreactivity, eosinophilia, and gross changes in pulmonary structure normally resulting from aeroallergen challenge were abolished, providing clear evidence of the central importance of IL-5 and eosinophils in aeroallergen-induced lung damage and airways hyperreactivity.

Materials and Methods

Aeroallergen Treatment of Mice. Mice (C57BL/6, 6–10 wk of age) were sensitized by i.p. injection with 50 μg OVA/1 mg Alhydrogel (CSL Ltd., 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 exposed to an aerosol of OVA (10 mg/ml) in 0.9% saline (nonsensitized mice received saline only) for 30 min three times (at 1-h intervals), and then every second day thereafter, for 8 d. The aerosol was generated at 6 liters/min by a nebulizer that produced a mean particle diameter of 3.9 μm into a closed chamber of 800 cm³. 3 h after the last aeroallergen challenge, mice were killed by

cervical dislocation, or airways hyperreactivity was measured. Lung responses of IL-5- mice were compared with control litter mates (IL-5+), which were simultaneously treated. Mice were treated according to Australian National University Animal Welfare guidelines and were housed in an approved containment facility.

Measurement of Airways Hyperreactivity. Mice were anesthetized with ketamine (60 mg/kg) and rompun (8 mg/kg) by intraperitoneal injection, and their trachea and tail vein were cannulated. The tracheal cannula was then connected to a rodent ventilator and a bronchospasm transducer (Ugo Basil 7020), which was coupled to a Lab Mac/8 analysis station (AdInstruments, Sydney, Australia). Mice were mechanically ventilated at 60 strokes/min, with a stroke volume of 1 ml after neuromuscular blockade with 0.5 mg/kg suxamethonium. Airways constriction was measured by determining changes in respiratory overflow volume during cumulative intravenous administration of β-methacholine. The increase in respiratory overflow volume provoked by β-methacholine is represented as a percentage of the maximal overflow volume (100%) obtained by totally occluding the tracheal cannula. To avoid an anesthesia-induced fall in body temperature, ambient temperature was maintained at 30°C.
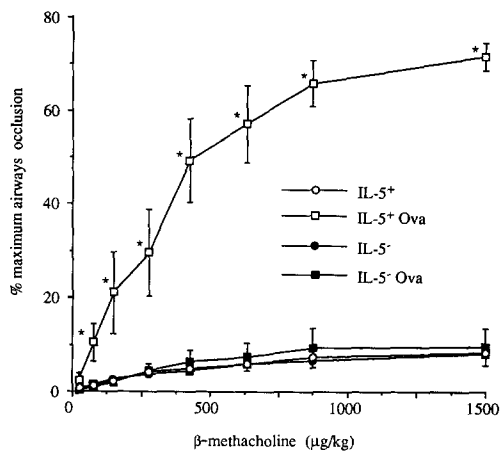
Figure 3. Measurement of airways reactivity to β-methacholine in nonsensitized and aeroallergen-challenged (OVA), sensitized IL-5+, and IL-5− mice. Airways constriction was measured by determining changes in respiratory overflow volume during cumulative intravenous administration of β-methacholine and is represented as a percentage of the maximal overflow volume (100%) obtained by totally occluding the tracheal cannula. Data represent the mean ± SEM for groups of five to seven 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) *$P < 0.001$ compared with responses of IL-5+ OVA. (b) *$P < 0.001$ compared with IL-5− OVA.

Histology. Lung tissue representing the central (bronchi-bronchiole) and peripheral (alveoli) airways was fixed in 10% phosphate-buffered formalin, sectioned, and stained with May–Grunwald–Giemsa solution or hematoxylin and eosin.

Collection and Analysis of Peripheral Blood and Bronchoalveolar Lavage Fluid. Tracheae were cannulated and the airways lumina were washed with 4 × 1 ml of 0.9% saline containing BSA (0.1% wt/vol) at 37°C. Approximately 0.8 ml of the instilled fluid was recovered per wash. The bronchoalveolar lavage fluid (BALF)[1] obtained from one animal was pooled and centrifuged (150 g) at 4°C for 10 min. Cell numbers were determined using a standard hemocytometer. BALF cells were resuspended in lymphocyte culture medium (100 μl), cytocentrifuged at 500 rpm for 5 min, and differentially stained with May–Grunwald–Giemsa solution. Cell types were identified using morphological criteria. 200–300 leukocytes were counted on each slide. Blood samples were drawn from the tail vein for differential cell counts.

Measurements of Serum IgE. Serum OVA-specific IgE was detected by isotype-specific ELISA using rat anti–mouse IgE mAb, clone LO-ME-3 (Biosource International, Camarillo, CA). OVA-specific IgE was quantified against standard mouse IgE. OVA-specific IgE was detected only at background levels (<10 ng) in unimmunized mice.

rVV Inoculation. rVV were constructed as described (27). Mice were given intranasal inocula of 10^7 PFU of VV-HA-TK or VV-HA-IL-5 in 20 μl saline under light ether anesthesia on days 24 and 28 after OVA sensitization. Virus growth in the lungs of mice given intranasal inocula of VV-HA-IL-5 or VV-HA-TK is sustained at similar levels over 6 d and is maximal at days 2–3. On day 30 after the last OVA aerosolization, lungs were removed for histological analysis.

Results and Discussion

Characterization of Allergic Airways Inflammation in Mice. Aerosol challenge of mice with OVA induced a significant increase in eosinophils in the lung (Fig. 1 *b*), blood (Fig. 2 *a*), and BALF (Fig. 2 *b*), in comparison to mice given aerosolized saline (Figs. 1 and 2). Eosinophilic inflammation was widespread, with dense cellular peribronchial and perivascular infiltration (Fig. 1, *b* and *c*). Infiltration of pulmonary lymphocytes and neutrophils was also significantly elevated (Fig. 2 *c*). Lung tissue in mice given OVA was characterized by gross alterations in the structural integrity of the airway walls (bronchi and broncheoli) and parenchyma, epithelial cell shedding, microvascular leakage and extensive mucosal edema, increased tissue cellularity, and particulate exudates in the airways lumina and alveolar septa (Fig. 1, *b* and *c*). In some animals, thickening of the epithelial basement membrane region and alterations in the integrity of the smooth muscle band were also observed (results not shown). Aeroallergen-induced pulmonary damage was directly associated with the induction of airways hyperreactivity to β-methacholine (Fig. 3).

The Role of IL-5 in Pulmonary Eosinophilia and Airways Dysfunction. The role of IL-5 in the selective accumulation of eosinophils and the development of pulmonary dysfunction was analyzed in IL-5− mice using the aeroallergen-induced lung damage model. IL-5− mice are specifically affected in IL-5 production, with no evidence of secondary effects on other cytokines or antibody production (26a). In marked contrast to the results with IL-5+ mice, OVA aerosolization of sensitized IL-5− mice of the C57BL/6 background induced no airways or blood eosinophilia (Fig. 2, *a*
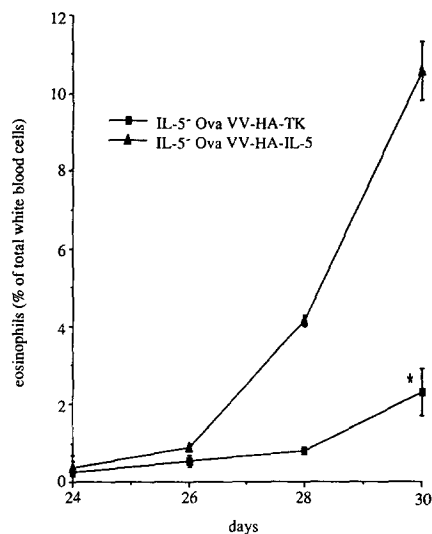

Figure 4. Eosinophil levels in peripheral blood from sensitized IL-5− mice infected with VV-HA-TK or VV-HA-IL-5 and exposed to aerosolized OVA. Data represent the mean ± SEM for groups of four mice. The significance of differences between experimental groups was analyzed using the unpaired Student's t test. Differences were considered significant if $P < 0.05$. *$P < 0.01$ compared with day 30 IL-5− OVA plus VV-HA-IL-5. Mice were inoculated with rVV, and levels of blood eosinophils were analyzed as described in Materials and Methods.

and *b*) and minimal morphological changes to pulmonary structure (Fig. 1, *d* and *e*). Lung histology and eosinophil numbers in BALF and blood taken from these animals resembled those of mice that were not actively sensitized (Figs. 1 *a* and 2, *a* and *b*). In contrast, neutrophil and lymphocyte numbers in BALF were significantly elevated after aeroallergen challenge of IL-5⁻ mice, although increases in the latter were less marked than in IL-5⁺ mice (Fig. 2 *c*).

OVA-specific IgE was detected at similar levels in sera from IL-5⁺ and IL-5⁻ mice after aeroallergen challenge (Fig. 2 *d*), indicating that IgE and eosinophilia are independently regulated, as previously shown in pulmonary parasitic infestation (28) and allergic bronchopulmonary aspergillosis (29). Airways hyperreactivity to β-methacholine was also abolished in aeroallergen-challenged IL-5⁻ mice (Fig. 3). These results establish that IL-5 plays a pivotal role in gen-
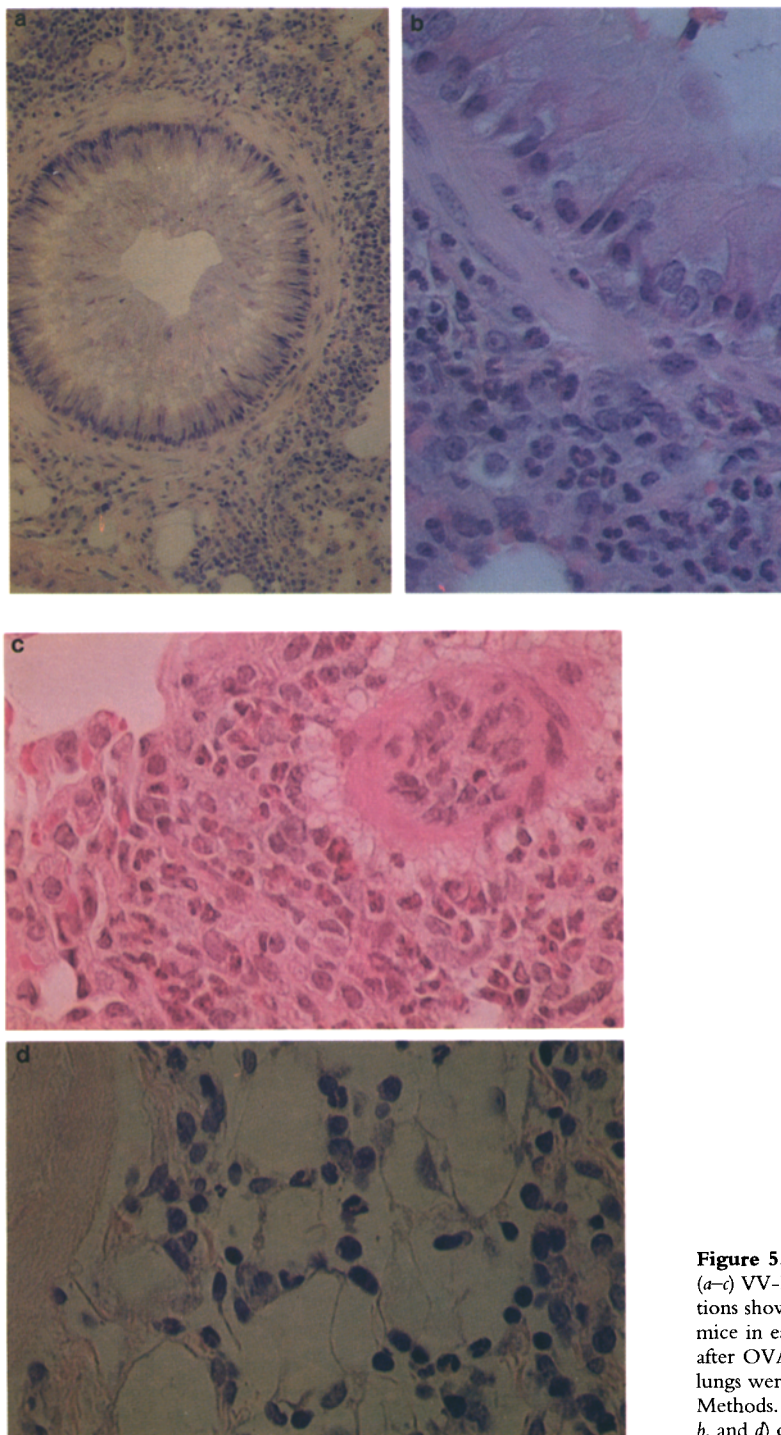
Figure 5. Histological analysis of lung sections from IL-5⁻ mice given (*a–c*) VV-HA-IL-5 and aerosolized OVA and (*d*) VV-HA-IL-5 only. Sections shown are representative of 10 sections of lung per mouse from five mice in each group. Mice were given intranasal inocula (days 24 and 28 after OVA challenge), and on day 30 after the last OVA aerosolization, lungs were removed for histological analysis as described in Materials and Methods. Sections were stained with May–Grunwald–Giemsa solution (*a*, *b*, and *d*) or hematoxylin and eosin (*c*). ×56 (*a*); ×214 (*b*); ×222 (*c–d*).
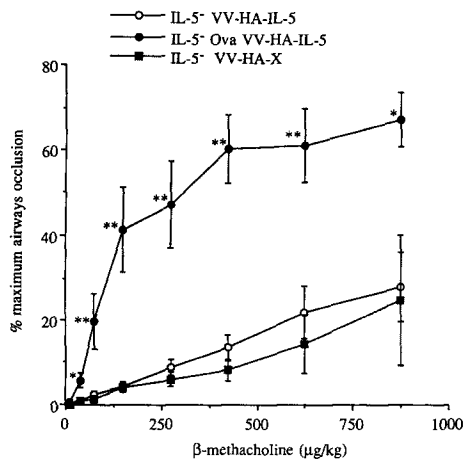
Figure 6. Measurement of airways reactivity to β-methacholine in aeroallergen-challenged (OVA), sensitized IL-5⁻ mice after reconstituting IL-5 production with VV-HA-IL-5. Airways constriction was measured by determining changes in respiratory overflow volume as described in Fig. 3. Data represent the mean ± SEM for groups of three to five 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. *P <0.05 and **P <0.01 compared with responses of IL-5⁻ VV-HA-IL-5.

erating the observed blood and airways eosinophilia and in the subsequent development of airways hyperreactivity and lung damage, which occurs in response to aeroallergens. These data also indicate that eosinophils are the proinflammatory cells primarily involved in the induction of changes in pulmonary structure and function. Histological analysis showed no increase in mast cell numbers after sensitization and aeroallergen challenge of IL-5⁺ or IL-5⁻ mice (results not shown), supporting previous evidence obtained from mast cell–deficient mice that this cell is not required for the induction of chronic airways inflammation in this model (30).

Restoration of Aeroallergen-induced Eosinophilia and Airways Dysfunction in IL-5⁻ Mice. The central role of IL-5 in aeroallergen-induced eosinophilic recruitment into the airways was confirmed by resconstituting IL-5 production in the lungs of IL-5⁻ mice by using a rVV expressing this factor (VV-HA-IL-5) (27). An appropriate control virus with-

out IL-5 (VV-HA-TK) was also used (27). rVV constructs encoding cytokine genes deliver these factors in a highly localized manner. The encoded cytokines are secreted from infected cells in amounts determined by the extent of viral replication, and have been shown to influence specific immune responses (27, 31). In the lungs of IL-5⁻ mice, VV-HA-IL-5 and VV-HA-TK localized in the parenchyma and foci of infected cells are established with similar tissue tropisms. The lungs of sensitized IL-5⁻ mice exposed to the control virus VV-HA-TK showed highly localized pockets of airways inflammation, which were not characterized by eosinophilic infiltration (results not shown) and which correlated with sites of virus infection. Exposure of these mice to aerosolized OVA did not induce further airways inflammation or lung damage. However, blood eosinophilia (Fig. 4), severe pulmonary eosinophilic inflammation and characteristic lung pathology (Fig. 5, *a–c*), and airways hyperreactivity (Fig. 6) were restored in IL-5⁻ mice challenged with aerosolized OVA and given VV-HA-IL-5. The lungs of IL-5⁻ mice given VV-HA-IL-5 in the absence of OVA sensitization and aerosolization showed some evidence of eosinophil migration, which was primarily localized to the sites of rVV infection but was not accompanied by dense cellular infiltration or changes in lung morphology (Fig. 5 *d*). It will be of interest to determine what factors derived from the site of OVA presentation are involved in the amplification of the signal for eosinophil migration and are essential for widespread eosinophilic inflammation and degranulation and the onset of pulmonary dysfunction. Recently, after stimulation with IgA, IgG, or IgE immune complexes, eosinophils were shown to degranulate and secrete significant amounts of IL-5 (32). The synthesis and secretion of IL-5 and other activating factors from eosinophils may provide an important autocrine pathway that regulates localized eosinophil accumulation and activation, and that modulates the inflammatory process.

We conclude that IL-5 is essential for mounting aeroallergen-induced pulmonary and blood eosinophilia, as well as the subsequent onset of airways hyperreactivity and lung damage in vivo. Our findings in a mouse asthma model strongly support clinical investigations that suggest that IL-5 is an important target for improved asthma therapy.

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