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

By Paul S. Foster,* Simon P. Hogan,* Alistair J. Ramsay,* Klaus I. Matthaei,* and Ian G. Young*

From the Divisions of*Biochemistry and Molecular Biology and *Cell Biology, John Curtin School of Medical Research, Australian National University, Canberra ACT 0200, Australia

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 \[\beta\]-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, leukotriene B4, IL-5, and chemokines [RANTES, macrophage chemoattractant protein, macrophage inflammatory protein, and eotaxin]) 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 B4, 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 relative 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).
aeroallergen-induced lung damage and airways hyperreactivity in vivo. In IL-5− mice, the airways hyperreactivity, eosinophils, 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 bronchospsms transducer (Ugo Basili 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.
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 bronchioles) 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 aerosolized 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

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) 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 107 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.

Abbreviations used in this paper: BALF, bronchoalveolar lavage fluid; rVV, recombinant vaccinia virus.
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).
The central role of IL-5 in aeroallergen-induced eosinophilic recruitment into the airways was confirmed by reconstituting IL-5 production in IL-5- mice after constituting 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.

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.

Address correspondence to Paul S. Foster, Division of Biochemistry and Molecular Biology, The John Curtin School of Medical Research, Australian National University, Canberra ACT 0200, Australia.

Received for publication 5 July 1995 and in revised form 28 August 1995.

References

1. Bochner, B.S., B.J. Undem, and L.M. Lichtenstein. 1994. Immunological aspects of allergic asthma. Annu. Rev. Immunol. 12:295–335.

2. De Monchy, J.G., H.F. Kauffman, P. Venge, G.H. Koeter, H.M. Jansen, H.J. Sluiter, and K. De Vries. 1985. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. Am. Rev. Respir. Dis. 131:373–376.

3. Beasley, R., W.R. Roche, J.A. Roberts, and S.T. Holgate. 1989. Cellular events in the bronchi in mild asthma and after bronchial provocation. Am. Rev. Respir. Dis. 139:806–817.

4. Azzawi, M., B. Bradley, P.K. Jeffery, A.J. Frew, A.J. Wardlaw, G. Knowles, B. Assoufi, J.V. Collins, S. Durham, and A.B. Kay. 1990. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma.

200 IL-5 Deficiency Abolishes Pulmonary Dysfunction
5. Ohashi, Y., S. Motojima, T. Fukuda, and S. Makino. 1992. Airway hyperresponsiveness, increased intracellular spaces of bronchial epithelium, and increased infiltration of eosinophils and lymphocytes in bronchial mucosa in asthma. *Am. Rev. Respir. Dis.* 145:1469–1476.

6. Walker, C., E. Bode, L. Boer, T.T. Hansel, K. Blaser, and J.C. Virchow, Jr. 1992. Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. *Am. Rev. Respir. Dis.* 146:109–115.

7. Robinson, D.S., Q. Hamid, S. Ying, A. Tsicopoulos, J. Barkans, A.M. Bentley, C. Corrigan, S.R. Durham, and A.B. Kay. 1992. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N. Engl. J. Med.* 326:298–304.

8. Walker, C., J.C. Virchow, Jr., P.L. Bruijnzeel, and K. Blaser. 1991. T cell subsets and their soluble products regulate eosinophilia in allergic and nonallergic asthma. *J. Immunol.* 146:1829–1835.

9. Wardlaw, A.J., S. Dunnette, G.J. Gleich, J.V. Collins, and A.B. Kay. 1988. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Relationship to bronchial hyperreactivity. *Am. Rev. Respir. Dis.* 137:62–69.

10. Broide, D.H., G.J. Gleich, A.J. Cuomo, D.A. Coburn, E.C. Federman, L.B. Schwartz, and S.I. Wannerman. 1991. Evidence of ongoing mast cell and eosinophil degranulation in symptomatic asthma. *J. Allergy Clin. Immunol.* 88:637–648.

11. Gleich, G.J., N.A. Flavahan, T. Fujisawa, and P.M. Vanhoutte. 1988. The eosinophil as a mediator of damage to respiratory epithelium: a model for bronchial hyperreactivity. *J. Allergy Clin. Immunol.* 81:776–881.

12. Sibille, Y., and H.Y. Reynolds. 1990. Macrophages and polymorphonuclear neutrophils in lung defense and injury. *Am. Rev. Respir. Dis.* 141:471–501.

13. Walker, C., M.K. Kacgi, P. Braun, and K. Blaser. 1991. Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. *J. Allergy Clin. Immunol.* 88:935–942.

14. Weller, P.F. 1994. Eosinophils: structure and functions. *Curr. Opin. Immunol.* 6:85–90.

15. Dahlen, C.A., T. Geier, T. Brunner, V. von Tschernar, D. Caput, P. Ferrara, A. Minty, and M. Baggiolini. 1994. Monocyte chemoattractant protein 3 is a most effective basophil-and eosinophil-activating chemokine. *J. Exp. Med.* 179:751–756.

16. Rot, A., T. Krieger, T. Brunner, S.C. Bischof, T.J. Schall, and C.A. Dahinden. 1992. RANTES and macrophage inflammatory protein 1a induce the migration and activation of normal human eosinophil granulocytes. *J. Exp. Med.* 176:1489–1495.

17. Resnick, M.B., and P.F. Weller. 1993. Mechanisms of eosinophil recruitment. *Am. J. Respir. Cell Mol. Biol.* 8:349–355.

18. Jose, P.J., D.A. Griffiths-Johnson, P.D. Collins, D.T. Walsh, R. Moqbel, N.F. Totty, O. Truong, J.J. Hsuan, and T.J. Williams. 1994. Eotaxin: a protein eosinophil chemotactic cytokine detected in a guinea pig model of allergic airways inflammation. *J. Exp. Med.* 179:881–888.

19. Lopez, A.F., C.J. Sanderson, J.R. Gamble, H.D. Campbell, I.G. Young, and M.A. Vadas. 1988. Recombinant human interleukin 5 is a selective activator of human eosinophil function. *J. Exp. Med.* 167:219–224.

20. Yamaguchi, Y., Y. Hayashi, Y. Sugama, Y. Miura, T. Kasa-