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Requirement for IL-13 Independently of IL-4 in Experimental Asthma

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Abstract

The pathogenesis of asthma reflects, in part, the activity of T cell cytokines. Murine models support participation of interleukin-4 (IL-4) and the IL-4 receptor in asthma. Selective neutralization of IL-13, a cytokine related to IL-4 that also binds to the α chain of the IL-4 receptor, ameliorated the asthma phenotype, including airway hyperresponsiveness, eosinophil recruitment, and mucus over-production. Administration of either IL-13 or IL-4 conferred an asthma-like phenotype to nonimmunized T cell-deficient mice by an IL-4 receptor α chain-dependent pathway. This pathway may underlie the genetic associations of asthma with both the human 5q31 locus and the IL-4 receptor.

Allergic asthma is a complex disorder characterized by local and systemic allergic inflammation and reversible airway obstruction. Asthma symptoms, especially shortness of breath, are primarily related to airway obstruction, and death is almost invariably due to asphyxiation (1). Increased airway responsiveness to provocative stimuli, termed airway hyperresponsiveness (AHR), and mucus hypersecretion by goblet cells are two of the principal causes of airway obstruction observed in asthma patients (2). Data from animal models consistently reveal a critical role for T_H2 (T helper 2) cells and potentially important roles for the cytokines IL-4 and IL-5 (3–7).

T_H2 cells selectively develop and expand in the presence of IL-4 (8). To separate direct effects of IL-4 from developmental effects on T_H2 cells in an asthma model, we compared the ability to establish the asthma phenotype in BALB/c mice deficient in either IL-4 or the IL-4 receptor α chain (IL-4R α) (9). After intranasal challenge with the antigen ovalbumin (OVA), BALB/c mice developed a stereotyped asthma phenotype characterized by eosinophil influx of the airways, goblet cell metaplasia with mucus overproduction, and an increase in AHR as revealed by enhanced sensitivity to acetylcholine challenge (6, 7). IL-4

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and IL-4R α -deficient mice showed incremental attenuation of each of these asthma indices (Fig. 1, C through E) (10). Thus, in agreement with prior studies (5–7), IL-4 contributes to the asthma phenotype, but these data suggest an independently greater contribution by IL-4R α .

IL-13 is a cytokine closely related to IL-4 that binds to IL-4R α and is also expressed by T_H2 cells from asthma patients (11). To assess whether IL-13 might contribute to the asthma phenotype, we administered a soluble IL-13 receptor α_2 -human Fc fusion protein (IL-13R-Fc) to BALB/c mice sensitized to OVA and compared them to mice that received control protein (12). IL-13R-Fc selectively binds to and neutralizes murine IL-13 but not IL-4 (13). This treatment significantly attenuated the asthma phenotype, although little effect was seen on neutrophil influx into bronchoalveolar lavage (BAL) (Figs. 1, E and F, and 2). Thus, IL-13, like IL-4 (5–7), can contribute to the acute effector phase of experimental asthma.

To assess the capacity of IL-13 and IL-4 to cause pathology independently of T and B cells, we administered each cytokine to nonimmunized BALB/c and RAG1 (recombinase activating gene 1)-deficient mice (14). Each cytokine alone induced the asthma phenotype (Figs. 1, A and B, and 3). In contrast, administration of either cytokine to IL-4R α -deficient mice resulted in no significant changes in any asthma parameter, demonstrating that their effects were dependent on signals mediated by IL-4R α . Further, adoptive transfer of OVA-specific T_H2 cells to IL-4R α -deficient mice failed to elicit the asthma phenotype, whereas identical treatment of wild-type mice resulted in the full phenotype (15, 16). Thus, experimental asthma induced by antigen challenge, recombinant cytokine, or adoptive transfer of T_H2 cells, is mediated through a final pathway dependent on IL-4R α .

Attenuated asthma phenotypes observed in IL-4-deficient mice may now be interpreted as representing the effects of residual IL-13 derived from IL-4-deficient T_H2 cells (17). Parallel observations in experimental intestinal helminth infections demonstrate roles for both IL-4 and IL-13 in mediating critical final effector pathways via IL-4R α (18). It is possible that human asthma represents a spectrum of disease also linked by a shared receptor effector pathway. The common embryological origin of tissues from the gut and lung (19) would support the presence of stereotyped responses in these organs.

The relevance of our data to human asthma remains an important issue that cannot be entirely addressed, given the complexity of the disease and the inadequacies of any animal model. Linkage analysis has mapped susceptibility to asthma to a region on human chromosome 5q25-31, which includes the genes for both IL-4 and IL-13 (20), and to mutations in two domains of the α chain of the IL-4 receptor (21). A number of additional regions in the genome have been linked to asthma in human studies, suggesting a complex multifactorial phenotype (22). As we suggest, however, diverse forms of asthma might follow a final common effector pathway mediated through signals transduced by IL-4R α , thus creating a unified target for potential intervention.

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References and Notes

1. Molfino NA, Nannini LJ, Martelli AN, Slutsky AS. N. Engl. J. Med. 1991; 324:285. [PubMed: 1986288]

2. Goldstein RA, Paul WE, Metcalfe DD, Busse WW, Reece ER. *Ann. Intern. Med.* 1994; 121:698. [PubMed: 7944080]
3. Gavett SH, Chen X, Finkelman F, Wills-Karp M. *Am. J. Respir. Cell Mol. Biol.* 1994; 10:587. [PubMed: 8003337]
4. Watanabe A, et al. *ibid.* 1997; 16:69. Garssen J, Nijkamp FP, Van Der Vliet H, Van Loveren H. *Am. Rev. Respir. Dis.* 1991; 144:931. [PubMed: 1833998] Rankin JA, et al. *Proc. Natl. Acad. Sci. U.S.A.* 1996; 93:7821. [PubMed: 8755560] Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. *J. Exp. Med.* 1996; 183:195. [PubMed: 8551223] Mauser PJ, et al. *Am. Rev. Respir. Dis.* 1993; 148:1623. [PubMed: 7504892]
5. Brusselle G, Kips J, Joos G, Bluethmann H, Pauwels R. *Am. J. Respir. Cell Mol. Biol.* 1995; 12:254. [PubMed: 7873190]
6. Corry DB, et al. *J. Exp. Med.* 1996; 183:109. [PubMed: 8551213]
7. Corry DB, et al. *Mol. Med.* 1998; 4:344. [PubMed: 9642684]
8. Abbas AK, Murphy KM, Sher A. *Nature.* 1996; 383:787. [PubMed: 8893001]
9. Wild-type, BALB/c IL-4^{-/-} (23), and C57BL/6 RAG1^{-/-} (24) mice were purchased from Jackson Laboratory. BALB/c IL-4Rα^{-/-} were obtained at the Max-Planck-Institut für Immunbiologie. Mice were immunized and intranasally challenged with chicken egg OVA (6, 7). AHR was expressed as the provocative concentration of acetylcholine (in milligrams per kilogram) that increased baseline airway resistance 200% (PC₂₀₀) (6, 7). Periodic acid-Schiff (PAS)-stained lung sections were examined at 250× magnification. Twenty to 50 consecutive airways from saline- and OVA-challenged mice were categorized according to the abundance of PAS⁺ goblet cells and assigned numerical scores (0: < 5% goblet cells; 1: 5 to 25%; 2: 25 to 50%; 3: 50 to 75%; 4: > 75%). The sum of the airway scores from each lung was divided by the number of airways examined for the histologic goblet cell score (expressed as arbitrary units; U). BAL samples were analyzed as described (7). Statistical significance was calculated using Student's *t* test (PC₂₀₀) or Wilcoxon test (goblet cell score, BAL cytology).
10. PC₂₀₀ of saline-treated controls was 0.55 ± 0.06 mg/kg. PC₂₀₀ of OVA-challenged wild-type, IL-4-deficient, and IL-4Rα-deficient mice were 0.24 ± 0.025, 0.36 ± 0.026, and 0.45 ± 0.058 mg/kg, respectively.
11. Minty A, et al. *Nature.* 1993; 362:248. [PubMed: 8096327] Punnonen J, et al. *Proc. Natl. Acad. Sci. U.S.A.* 1993; 90:3730. [PubMed: 8097323] Naseer T, et al. *Am. J. Respir. Crit. Care Med.* 1997; 155:845. [PubMed: 9117015] Till S, et al. *Immunology.* 1997; 91:53. [PubMed: 9203965]
12. After subcutaneous priming with OVA on days -14 and -7, BALB/c wild-type mice were administered intranasally 55 µl of IL-13R-Fc (13) or immunoglobulin control protein (Ig control) in phosphate-buffered saline (PBS). We administered 110 µg of IL-13R-Fc on days 0, 1, 2, 3, 3.5, 4, and 4.5, and 110 µg of Ig control was given on days 0, 1, 2, and 4. Both proteins were administered with 750 µg of OVA on days 1, 2, and 4. Data were collected on day 5 (9).
13. Donaldson DD, et al. *J. Immunol.* 1998; 161:2317. [PubMed: 9725226]
14. Wild-type, IL-4Rα^{-/-}, and RAG1^{-/-} mice were administered 3 to 5 µg of recombinant IL-4 or IL-13, or control protein (bovine serum albumin or irrelevant rat antibody) intranasally on days 1, 3, and 5, and data were collected 12 to 15 hours later (9). For clarity, only data from BALB/c wild-type mice are shown.
15. CD4⁺ T cell lines were prepared using splenocytes from D011.10 T cell receptor (TCR) transgenic mice that express an OVA-specific TCR transgene (25). Equal numbers of T cells and antigen presenting cells (mitomycin c-treated and T cell-depleted BALB/c splenocytes) were incubated with 1 µM OVA peptide, IL-4 (300 IU/ml), and antibody to IFN-γ (R46A2; 100 µg/ml) for 5 days. Wild-type or IL-4Rα^{-/-} mice were reconstituted with 1.2 × 10⁷ washed cells intravenously. The mice were challenged intranasally with OVA for 6 consecutive days. Data were collected on day 7 (9).
16. Grünig G, Corry DB. unpublished data.
17. Hogan SP, et al. *J. Immunol.* 1998; 161:1501. [PubMed: 9686617] Cohn L, Homer RJ, Marinov A, Rankin J, Bottomly K. *J. Exp. Med.* 1997; 186:1727.
18. Bancroft AJ, McKenzie AN, Grecis RK. *J. Immunol.* 1998; 160:3453. [PubMed: 9531306] Urban JF Jr, et al. *Immunity.* 1998; 8:255. [PubMed: 9492006] Urban JF Jr, Katona IM, Paul WE,

- Finkelman FD. Proc. Natl. Acad. Sci. U.S.A. 1991; 88:5513. [PubMed: 2062833] Barner M, Mohrs M, Brombacher F, Kopf M. Curr. Biol. 1998; 8:669. [PubMed: 9635196]
19. Ten Have-Opbroek AA. Exp. Lung Res. 1991; 17:111. [PubMed: 2050021]
20. Postma DS, et al. N. Engl. J. Med. 1995; 333:894. [PubMed: 7666875]
21. Mitsuyasu H, et al. Nature Genet. 1998; 19:119. [PubMed: 9620765] Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. N. Engl. J. Med. 1997; 337:1720. [PubMed: 9392697]
22. Daniels SE, et al. Nature. 1996; 383:247. [PubMed: 8805698] Sandford A, Weir T, Pare P. Am. J. Respir. Crit. Care Med. 1996; 153:1749. [PubMed: 8665031]
23. Noben-Trauth N, Kohler G, Burki K, Ledermann B. Transgenic Res. 1996; 5:487. [PubMed: 8840532]
24. Mombaerts P, et al. Cell. 1992; 68:869. [PubMed: 1547488]
25. Murphy KM, Heimberger AB, Loh DY. Science. 1990; 250:1720. [PubMed: 2125367]

Fig. 1. PAS-stained histologic sections of murine lungs. Arrowheads point to goblet cells within the respiratory epithelium. (A) Wild-type mice were primed with OVA and challenged with PBS intranasally. (B) Wild-type mice were administered IL-13 intranasally. (C) IL-4-deficient and (D) IL-4R α -deficient mice were primed with OVA and challenged with OVA intranasally. Wild-type mice were primed with OVA and challenged intranasally with (E) OVA and human Fc control protein or with (F) OVA and IL-13R-Fc. Note the marked reduction in goblet cells in (D) and (F).

Fig. 2. Effect of neutralization of IL-13. Primed wild-type mice were administered intranasally human immunoglobulin (Ig control), Ig control and OVA, or IL-13R-Fc and OVA as indicated by (+). Data for (A) AHR, (B) goblet cell score, and numbers of (C) eosinophils and (D) neutrophils in the BAL fluid are plotted as means $\pm$ SEM. * $P < 0.05$ relative to PBS and Ig control-treated mice; † $P < 0.05$ relative to OVA and Ig control-treated mice. Data are representative of at least two comparable experiments with four to eight mice per group.

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Fig. 3. Effect of recombinant IL-4 and IL-13. Wild-type (WT), RAG1-deficient (RAG1^{-/-}), and IL-4Rα-deficient (IL-4Rα^{-/-}) mice were administered IL-4, IL-13, or control protein intranasally. Data for (A) AHR, (B) goblet cell score, and numbers of (C) eosinophils and (D) neutrophils in the BAL fluid are plotted as means ± SEM. **P* < 0.05 relative to mice receiving control protein. Data are representative of at least two comparable experiments with four to eight mice per group.