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Requirements for Allergen-Induced Airway Hyperreactivity in T and B Cell–Deficient Mice

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Abstract

Background: The pathogenesis of asthma is believed to reflect antigen-induced airway inflammation leading to the recruitment of eosinophils and activation of mast cells through cell-associated IgE. Controversies persist however, regarding the relative importance of different pathogenic cells and effector molecules.
Materials and Methods: A variety of gene-targeted mice were examined for the induction of cholinergic airway hyperresponsiveness (AH), allergic airway inflammation, mucus production, and serum IgE reactivity following intratracheal challenge with a potent allergen. AH was determined using whole-body plethysmography following acetylcholine challenge. Where possible, results were confirmed using neutralizing antibodies and cell-specific reconstitution of immune deficient mice.
Results: T and B cell–deficient, recombinase-activating-gene-deficient mice (RAG −/−) failed to develop significant allergic inflammation and AH following allergen challenge. Reconstitution of RAG −/− mice with $CD4^+$ T cells alone was sufficient to restore allergen-induced AH, allergic inflammation, and goblet cell hyperplasia, but not IgE reactivity. Sensitized B cell–deficient mice also developed airway hyperreactivity and lung inflammation comparable to that of wild-type animals, confirming that antibodies were dispensable. Treatment with neutralizing anti-IL-4 antibody or sensitization of IL-4-deficient mice resulted in loss of airway hyperreactivity, whereas treatment with anti-IL-5 antibody or sensitization of IL-5-deficient mice had no effect.
Conclusions: In mice, $CD4^+$ T cells are alone sufficient to mediate many of the pathognomonic changes that occur in human asthma by a mechanism dependent upon IL-4, but independent of IL-5, IgE, or both. Clarification of the role played by $CD4^+$ T cells is likely to stimulate important therapeutic advances in treatment of asthma.

Introduction

Asthma is a complex clinical disorder marked by reversible episodes of airway narrowing and inflammation. Inflammation is characterized by the presence of Th2 lymphocytes that produce a spectrum of cytokines, including interleukin-3 (IL-3), IL-4, IL-5, IL-10, IL-13, and granulocyte-macrophage colony-stimulating factor (GM-CSF) (1–5). Together, these cytokines are believed to mediate the recruitment and activation of eosinophils (by IL-5 and GM-CSF) and mast cells (by IL-3, IL-4, IL-9, IL-10, and IL-13) and

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the production of IgE antibodies (by IL-4 and IL-13) that ultimately cause pathologic changes, including damage and sloughing of the respiratory epithelium, excess mucus production, airway edema, and changes in airway smooth muscle that collectively lower the threshold for airway narrowing to a variety of stimuli, termed airway hyperresponsiveness (AH) (6,7).

Recently, mouse models of allergic airway inflammation have been used to identify host determinants involved in the inflammatory and physiologic changes associated with human asthma. As expected, T cell–deficient mice failed to develop lung inflammation and AH (8–11), and the role for Th2 cells predicted by the human studies was validated using anti-IL-4 and anti-IL-4 receptor antibodies and in IL-4-deficient mice, which failed to develop antigen-induced airway changes in most (8,12,13), but not all (14), studies. Activity mediated in the absence of IL-4 may reflect the capacity of IL-13, which can be coordinately expressed with IL-4 in asthma (15), to itself direct Th2 differentiation through STAT6-mediated stimulation in some strains of mice (16). Mice engineered to express excessive amounts of IL-4 in the lung developed tissue inflammation, including eosinophils, and baseline elevations of pulmonary resistance, but they did not develop AH, indicating additional requirements for the full spectrum of disease (17). Roles for eosinophils, IL-5 and IgE in AH have been more variable, with some studies suggesting a necessary role and others demonstrating one or all to be dispensable (reviewed in refs. 18, 19, and discussed below).

The fungus *Aspergillus fumigatus* has been shown to be a potent pulmonary allergen (20) and is known to cause allergic airways disease in humans (21). We used an extract of Aspergillus that induced AH across a range of inbred mouse strains to investigate the critical components of the host response required to generate AH in the mouse. AH was rigorously quantitated in the intact animal while breathing at physiologic tidal volumes. As assessed in both $CD4^+$ T cell–reconstituted, recombinase-activating-gene (RAG)–deficient mice and in B cell–deficient mice, T cells were sufficient to mediate acute AH and lung infiltration by inflammatory cells. Furthermore, depletion of eosinophils to baseline levels had no effect on the physiologic or immunologic changes mediated by $CD4^+$ T cells. IL-4, but not IL-5, emerged as a critical effector molecule mediating AH. This model should be useful in elucidating the minimal signals required to elicit allergen-induced lung disease.

Materials and Methods

Mice

Female C57BL/6 (Jackson Laboratories, Bar Harbor, ME) and 129Sv/Ev (Taconic Laboratories, Germantown, NY) mice were used at 6–10 weeks of age. Mice rendered deficient in B cells by targeted disruption of the membrane exon of the immunoglobulin μ chain gene (22) and mice rendered deficient in T and B cells by disruption of the recombinase activating gene-1 (RAG-1) (23) were purchased from Jackson Laboratories and backcrossed six times to C57BL/6 mice. RAG-2-deficient mice (24) were on the 129SvEv background (Taconic Laboratories), IL-4-deficient mice (25) were on the 129Sv/Ev background, and IL-5-deficient (26) mice were on the C57BL/6 background.

Antigen sensitization

Aspergillus fumigatus antigens represent a mixture of culture filtrate and mycelial extract prepared free of living organisms (27). Batches #5323R1 and 5325 with equivalent activities were aliquoted at 10 mg/ml and stored at −70°C prior to use. The endotoxin content of the extract was less than 0.1 EU/100 μg antigen.

For sensitization, anesthetized mice were given 100 μg *Aspergillus* extract in 50 μl phosphate-buffered saline (PBS) intranasally five times at 4-day intervals as described previously (20) or, with slight modification, by first systemically priming animals with three subcutaneous injections of 100 μg *Aspergillus* extract in 50 μl PBS at 4-day intervals, followed by delivery of the same amount of antigens three times at 4-day intervals to anesthetized animals via a blunt-end gavage needle inserted into the upper airway by direct visualization. No physiologic or pathologic differences occurred among mice immunized by either of the two protocols (D. B. Corry and G. Grünig, unpublished data). Designated mice were given 2 mg of neutralizing anti-murine IL-5 antibody, TRFK-5 (28), or 2 mg of neutralizing anti-murine IL-4 antibody, 11B11 (29), intraperitoneally, beginning on the first day of antigen sensitization and then concurrently with additional antigen administration every 4 days. Control mice were given an isotype-matched irrelevant monoclonal antibody, Y13-259.

Quantitation of AH

Mice were anesthetized and maintained inside a whole-body plethysmograph on rodent ventilators as described previously (8). For convenience, animals were studied 4 days after the final antigen challenge, although entirely comparable results occurred when mice were investigated either immediately or 1 day after the final antigen challenge (D. Corry and G. Grünig; unpublished observations). Briefly, mice were ventilated using 100% oxygen under conditions that maintained physiologic pH and PCO_2. It was not possible to maintain physiologic conditions if mice were ventilated with room air, because of the severe hypoxemia incurred after administration of the higher doses of acetylcholine (D. Corry, unpublished data). After establishing a stable baseline for total lung resistance (R_L) as determined by continuously quantitating $\Delta Pt/\Delta V$ (where ΔPt = change in tracheal pressure and ΔV = change in flow) at 70% tidal volume, acetylcholine chloride was administered intravenously over 1 sec in escalating doses via an indwelling tail vein catheter. The provocative concentration of acetylcholine in μg/gm that caused a 200% increase in R_L, designated PC_{200}, was calculated by linear interpolation of appropriate dose–response curves. Significant differences (defined as $p < 0.05$) were calculated on the logarithm of PC_{200} by analysis of variance using reference to the specified control groups. Baseline pulmonary resistance did not differ among the various groups of mice in these studies.

Assessment of the immune response

Bronchoalveolar lavage (BAL) cells were collected by serially instilling and withdrawing 1-ml aliquots of Hanks' balanced salt solution (HBSS), pH 7.2, from the tracheal cannula. Cells were washed, counted, and adjusted to 10^7 cells/ml in RPMI 1640 with 5% fetal bovine serum (FBS) and antibiotics. Aliquots of 10^5 cells were centrifuged onto glass slides, stained using modified Giemsa, and used to determine the absolute numbers of eosinophils.

Suspensions of lung cells were prepared by removing the whole lungs and dissecting away lymph node and thymic tissue. Lungs were finely minced and the fragments were pressed through a 0.75-μm nylon mesh filter. Red blood cells were lysed in hypotonic buffer, and the remaining cells were washed twice, counted, and adjusted to 10^7 cells/ml in RPMI 1640 with 5% FBS and antibiotics.

The numbers of IL-4-producing cells were quantitated using enzyme-linked immunocell spot (ELISPOT) assays as described previously (8). Briefly, duplicate cell samples were distributed to 96-well microtiter plates that had been precoated with mAb 11B11 anti-murine-IL-4 antibody, serial 2-fold dilutions of the cells were carried out, and the plates were incubated undisturbed for 18 hr at 37°C. After washing away the cells, biotinylated secondary antibody against IL-4, BVD6-24G.2, was added. Captured IL-4 was revealed using streptavidin-conjugated alkaline phosphatase and developed using 5-bromo-4-chloro-indolyl-phosphate in 0.1 M 2-amino-2-methyl-1-propanol buffer suspended in 0.6% agarose. Individual blue spots were counted after solidification of the agar using inverted microscopy.

Serum was prepared from whole blood collected at the time of death for determination of total IgE and IgG levels using sandwich monoclonal antibody-based ELISA as described elsewhere (8).

Tissue pathology

For histologic analysis, whole lungs were infused via the trachea with 4% formalin until distended and fixed for 24 hr. The tissues were embedded in paraffin, and 2- to 3-μm sections were cut and stained with hematoxylin and eosin or periodic acid-Schiff for viewing by light microscopy.

T cell reconstitutions

Spleen, inguinal, and axillary lymph nodes were harvested from C57BL/6 or 129SvEv mice. The tissues were finely minced and dispersed into single-cell suspensions by passage through a 0.75-μm nylon mesh filter. Cells were washed twice, counted, and depleted of B cells, MHC class II- and CD8-bearing cells using monoclonal antibodies J11d, BP107, and 3.155 (all from American Type Culture Collection; Rockville, MD), respectively, and low-toxicity rabbit and guinea pig complement (Cedarlane; Ontario, Canada). The resulting cells were labeled with phycoerythrin (PE)-conjugated anti-B220, FITC-conjugated anti-Thy 1.2, and Tri-Color-conjugated anti-CD4 and sorted on a flow cytometer (FACS Star Plus, Becton and Dickinson; Mountain View, CA) to achieve final populations of 96–98% pure $CD4^+$, $Thy1^+$, $B220^-$ lymphocytes. $CD4^+$ T cells were transferred to RAG-1 −/− (C57BL/6 donor cells) or RAG-2 −/− (129 SvEv donor cells) mice intraperitoneally in 0.5

ml RPMI 1640. The next day and every 4 days thereafter, mice were sensitized with 100 μg *Aspergillus* antigens in 50 μl PBS or PBS alone for 3 doses given subcutaneously followed by 3 doses given intranasally. At the conclusion of the experiment, aliquots of lung and spleen cells were analyzed for the presence of $CD4^+$, $CD8\alpha^+$, IgM^+, and $CD19^+$ cells using the appropriate conjugated monoclonal antibodies and flow cytometry.

Results

Airway responses induced by Aspergillus *antigens require lymphocytes*

After sensitization with antigens from *Aspergillus*, control mice (C57BL/6 or 129), but not RAG-1- or RAG-2-deficient mice that lacked T and B cells, developed substantial increases in airway hyperreactivity, as demonstrated by the significant decrease in the dose of acetylcholine, a potent airway constrictor, required to elicit a 200% increase in pulmonary resistance (provocative dose 200%, or PC_{200}) (Fig. 1). RAG-deficient mice also failed to develop the degree of airway inflammation—airway eosinophils and increases in the numbers of IL-4-producing cells in the lung—that characterized the response in wild-type mice, although some increases in BAL eosinophils could be consistently shown (Table 1). The inflammatory changes were markedly suppressed compared with those in wild-type mice, however, as revealed in multiple histologic sections from lungs, and this did not differ between RAG-1 −/− and RAG-2 −/− mice (Fig. 2). Thus, lymphocytes were required to mediate both the physiologic and pathologic changes that occured in the airways of mice challenged with the fungal antigens.

RAG-deficient mice reconstituted with $CD4^+$ T cells manifest allergen-induced physiologic and pathologic findings in the lungs

To confirm that T cells alone, and particularly $CD4^+$ T cells, could mediate the pathologic changes associated with asthma, spleen and lymph node $CD4^+$ T cells from the appropriate matched donor animals were purified by flow cytometry and were passively transferred into RAG-1- or RAG-2-deficient mice. The reconstituted mice were sensitized with antigen or saline, and the pathologic and physiologic changes were assessed. In contrast to nonreconstituted

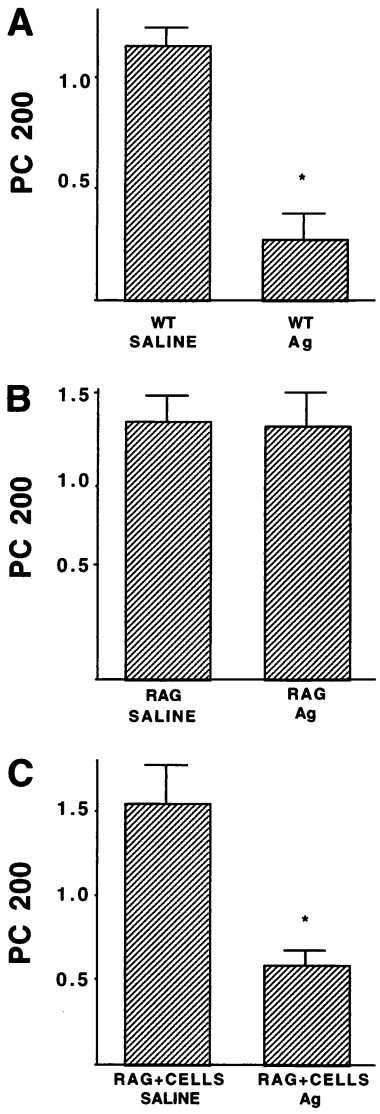

Fig. 1. Provocative concentrations of acetylcholine (μg/gm) that increased baseline airway resistance 200% (PC_{200}). Groups of 4–18 C57BL/6 (wild-type, WT) (A), RAG-1-deficient (RAG) (B) and RAG-1- or RAG-2-deficient mice reconstituted with purified $CD4^+$ T cells (RAG + Cells) (C) were sensitized with either saline (Saline) or *Aspergillus* antigens (Ag). Decreases in PC_{200} indicate enhanced airway hyperreactivity, with bars representing means and standard errors of the means. Significant differences ($P < 0.05$) between control and Ag groups are indicated (*).

RAG-deficient mice, mice reconstituted with $CD4^+$ T cells developed AH to acetylcholine (Fig. 1), as well as airway eosinophilia, tissue IL-4-producing cells, and peribronchiolar inflammatory changes in the lung (Table 1 and Fig. 2). Flow cytometric analysis confirmed the presence

Table 1. Immune responses to inhaled *Aspergillus* antigens

Mice	Total IL-4-Producing Cells/Lung	No. Eosinophils in BAL Fluid (%)	IgE (μg/ml)
C57BL/6			
Saline	130	0.8 ± 0.5 (1)	<0.1
Aspergillus	1692 ± 356	295 ± 27* (69)	4.5 ± 1.4*
RAG −/−			
Saline	<30	0.5 ± 0.4 (1)	<0.04
Aspergillus	<30	53 ± 13* (21)	<0.04
RAG −/− + CD4 T cells			
Saline	<30	26 ± 6 (41)	<0.04
Aspergillus	2400 ± 1400*	335 ± 50* (77)	<0.04
μMT			
Saline	208 ± 130	3 ± 2 (1)	<0.04
Aspergillus	614 ± 137*	259 ± 31* (65)	<0.04
Aspergillus + anti-IL-5	N.D.	6 ± 2 (2)	<0.04
Aspergillus + anti-IL-4	N.D.	128 ± 81* (30)	<0.04
IL-5 −/−			
Saline	N.D.	0 (0)	<0.04
Aspergillus	N.D.	2.7 ± 0.8 (6)	5.6 ± 0.5*

Groups of 4–18 mice from the designated groups were used to prepare single-cell suspensions of lung tissue for quantitation of numbers of IL-4-secreting cells by ELISPOT assay. Bronchoalveolar lavage (BAL) cells were stained to assess the absolute numbers ($\times 10^4$) and percentages (in parentheses) of eosinophils in total BAL cells. Serum IgE was quantitated by ELISA. Numbers represent mean ± SEM, with significant differences between saline and *Aspergillus* challenge indicated ($*p < 0.05$). N.D., not done.

of $CD4^+$ and the absence of $CD8^+$ T cells and $CD19^+$ B cells in the reconstituted, but not the nonreconstituted, RAG-deficient mice (Fig. 3). Serum immunoglobulins (IgG, IgE) were below the limits of detection of the ELISA in these reconstituted mice, suggesting that T cells mediated these changes in the absence of antibodies, including IgE.

Allergen-sensitized B cell–deficient mice develop AH and lung inflammation

To confirm that antibodies were not required to mediate AH, mice with targeted disruption of the immunoglobulin μ heavy chain gene, which are deficient in B cells and antibody (21), were immunized with *Aspergillus* antigens. These sensitized B cell–deficient mice developed AH, as quantitated by decreases in PC_{200}, that was comparable to that seen in B cell–replete mice (Fig. 4). Moreover, eosinophil recruitment to the airways and tissues, the appearance of lung Th2 cells, the degree of peribronchial/peribronchiolar inflammation, and the presence of goblet cell hyperplasia were all preserved in the sensitized B cell–deficient mice (Table 1 and Fig. 2). Flow cytometric analysis of spleen cells recovered at the end of the experiments, as well as serum immunoglobulin determinations, confirmed that these animals lacked B cells and antibody.

IL-4, but not eosinophil recruitment mediated by IL-5, is required to mediate AH

In prior experiments using mice sensitized with ovalbumin, we demonstrated requirements for IL-4, but not IL-5, using neutralizing antibodies administered during the period of antigen priming (8). Similarly, neutralization of IL-4 during the period of antigen sensitization in B cell–deficient mice completely abolished the development of AH. Eosinophil recruitment to the lung was diminished by approximately 50% in these mice (Fig. 4 and Table 1). In contrast, administration of anti-IL-5 antibody such that eosinophils were reduced to levels seen in nonimmu-

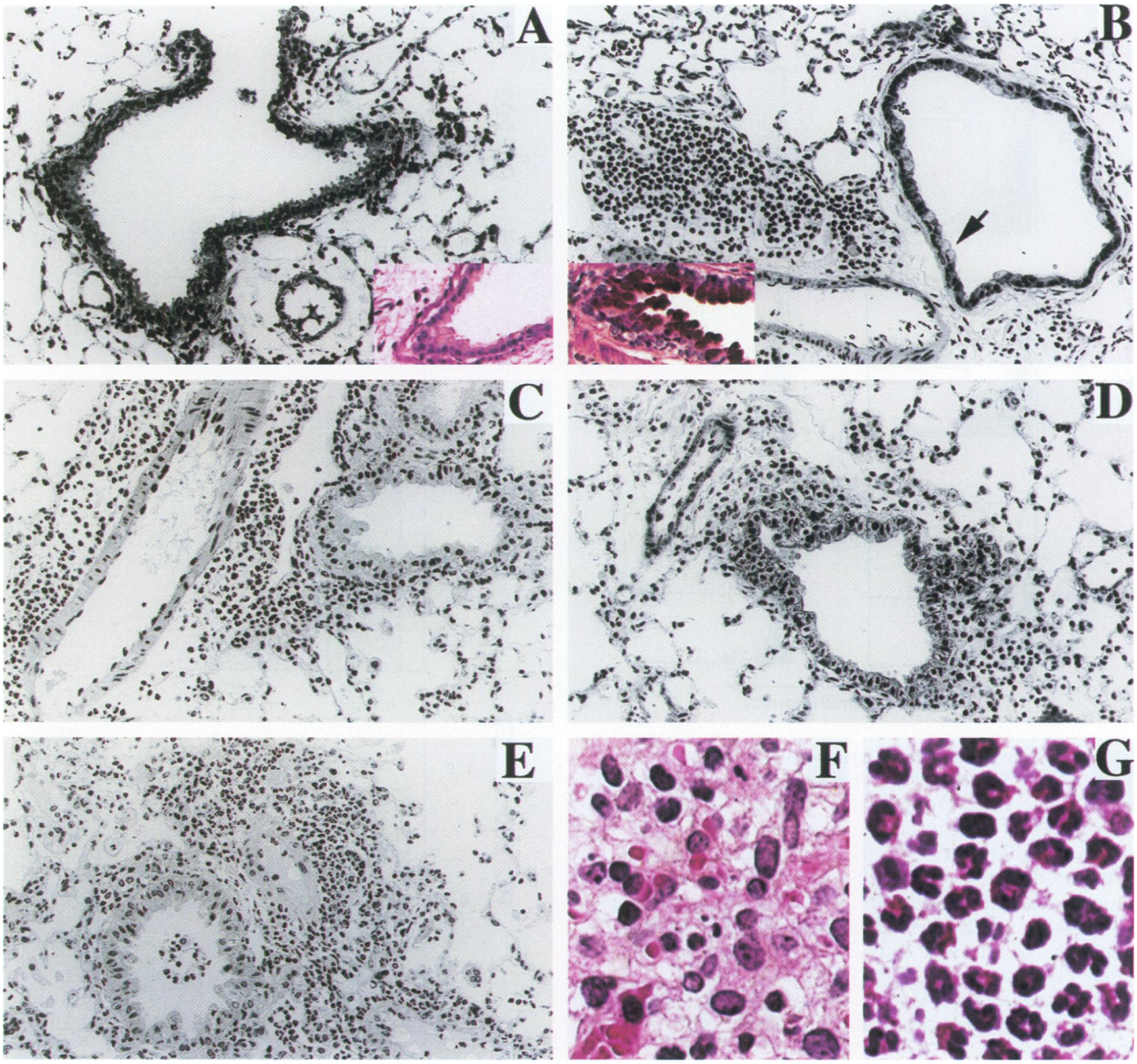

Fig. 2. Photomicrographs of representative tissue sections prepared from lungs of designated mice 4 days after the final antigen challenge. (A) μMT lung sensitized with saline. Minimal lymphoid cell infiltrate is apparent in the periarterial space adjacent to a normal bronchiole. Alveolar spaces are not involved. This was not different from that seen in C57BL/6 mice sensitized with saline. Inset: PAS staining reveals absence of goblet cells in epithelial cell layer. (B) C57BL/6 lung sensitized with *Aspergillus* extract. Periarteral space is filled with an eosinophil-rich infiltrate. Airway contains many pale-staining, vacuolated goblet cells (arrow). Inset: PAS stain highlights hyperplasia of purple-staining goblet cells in epithelial cell layer. (C) μMT lung sensitized with *Aspergillus* extract. The periarterial space is filled with an eosinophilic inflammatory infiltrate that spreads to the adjacent bronchiole. (D) μMT lung sensitized with *Aspergillus* extract and treated with anti-IL-5 antibody. The perivascular and peribronchiolar spaces are filled with a lymphoid infiltrate containing only rare eosinophils. (E) RAG-deficient lung reconstituted with $CD4^+$ T cells and sensitized with *Aspergillus* extract. A heavy perivascular infiltrate with eosinophils is present surrounding the peribronchiolar space. (F) High-power field of D demonstrating marked reduction in numbers of eosinophils after treatment with anti-IL-5 antibody. (G) High-power field of E demonstrating homogeneous eosinophil-rich infiltrate. All sections stained with hematoxylin and eosin. A–E, × 250 magnification; F, G ×1500 magnification.

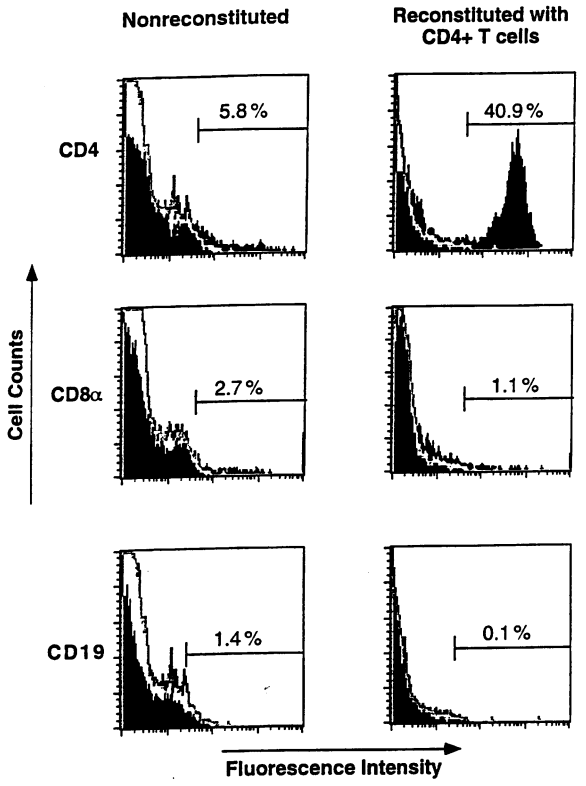

Fig. 3. Flow cytometric analysis of spleen cells from CD4$^+$ T cell–reconstituted RAG-deficient mice. Spleen cells from RAG-1- or RAG-2-deficient mice that had been either untreated (Nonreconstituted) or repleted with purified CD4$^+$ T cells (Reconstituted with CD4$^+$ T cells) were stained with fluorescence-conjugated monoclonal antibodies to CD4, CD8α, or CD19 (black shaded areas) to mark T helper and T cytotoxic cells and B lymphocytes, respectively. Gates were adjusted such that background staining with isotype control-matched fluorescence-conjugated monoclonal antibody (gray lines) was <3%.

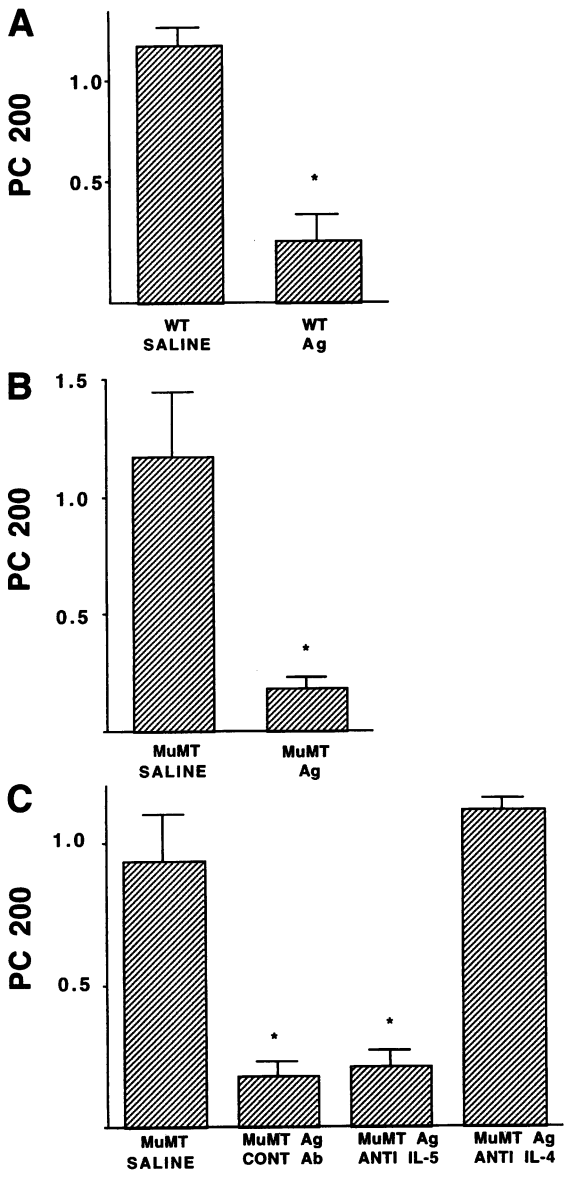

Fig. 4. Airway hyperreactivity in B cell–deficient mice. Groups of 4–18 C57BL/6 wild-type (WT) (A) or B cell–deficient (MuMT) mice (B) were sensitized with either saline (Saline) or *Aspergillus* antigens (Ag). Designated groups of MuMT mice were treated with anti-IL-5, anti-IL-4, or control (CONT Ab) monoclonal antibodies throughout the sensitization period (C). The provocative concentrations of acetylcholine (μg/gm) that increased the baseline airway resistance by 200% (PC_{200}) are represented by bars and standard errors of the means. Significant differences ($P < 0.05$) between the antigen and control groups are indicated (*).

nized control animals had no effect on the induction of AH (Table 1, Figs. 2 and 4).

To further confirm the IL-4 dependence and IL-5 independence of the airway phenotype mediated by sensitization with *Aspergillus* antigens, IL-4-deficient and IL-5-deficient mice were sensitized and analyzed for AH. Cohorts of wild-type mice were analyzed concurrently. Although the wild-type and IL-5-deficient mice had comparable induction of AH by the stringent physiologic criteria used, the IL-4-deficient mice had no airway reactivity (Fig. 5). As anticipated, sensitized IL-5-deficient mice did not substantially increase the numbers of eosinophils recovered in BAL compared with wild-type mice, but generated levels of IgE that were comparable to those in control animals (Table 1).

Discussion

These studies demonstrate that T cells, in the absence of B cells, IgE, or elevations in the num-

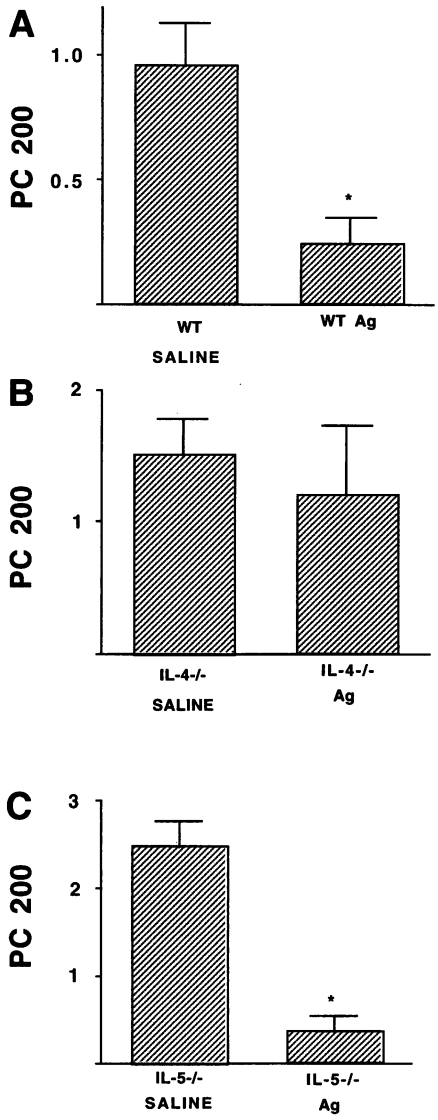

Fig. 5. Allergen-induced airway disease in cytokine-deficient mice. Cohorts of 4–6 wild-type (WT), IL-4-deficient (IL-4 −/−) or IL-5-deficient (IL-5 −/−) mice were sensitized five times intranasally with either saline (Saline) or *Aspergillus* antigens (Ag). Four days after the last intranasal challenge, mice were anesthetized and the airway resistance quantitated as the PC_{200}, which is represented by bars and standard errors of the means. Significant differences ($p < 0.05$) between the saline and Ag groups are indicated (*).

bers of eosinophils, were capable of inducing acute AH in mice sensitized to a potent airway allergen. Furthermore, the recruitment of eosinophils and the establishment of tissue inflammation were unimpeded by the absence of B cells and antibody. These studies are in accord with prior findings regarding inflammation in B cell–deficient mice by investigators using different antigenic challenges (30,31), but they extend these observations by the concomitant evaluation of pulmonary resistance in vivo. We also noted marked hyperplasia of goblet cells, an additional histologic hallmark of asthmatic airways (32), in antigen-sensitized, B cell–deficient mice. At least in this model, inflammation induced by the cross-linking of immunoglobulin Fc receptors on mast cells or eosinophils was not required to establish these key pathologic and physiologic components that occur in human asthma. Although eosinophils and mast cell–associated IgE undoubtedly contribute to the chronicity of human disease through their own elaboration of type 2 cytokines and other toxic factors (6,7,33–39), these data establish that $CD4^+$ T cells, in an IL-4-dependent manner, were alone capable of initiating the process, an important observation that may be relevant to therapeutic strategies applicable to human disease. This extends earlier reports indicating the capacity to transfer AH in rodents using sensitized $Thy\text{-}1^+$ or $CD4^+$ lymphocytes adoptively given into animals with otherwise intact immune systems (40,41), by rigorously defining the capacity of helper T cells to alone mediate the key pathologic and histologic markers of airway disease.

Despite intensive study, the role of IgE and eosinophils, both in murine models of allergen-induced lung disease and in human asthma, remains controversial. Studies in B cell–deficient and IgE-deficient mice have demonstrated that IgE is not required to mediate pulmonary inflammation, including the recruitment of eosinophils, in allergen-induced airways disease (30,42,43). In contrast, other investigators have reported a critical role for IgE in eosinophil recruitment as studied using anti-IgE antibodies (44) or in analysis of hyper-IgE-producing lines of mice (45), or in inducing tracheal smooth muscle hyperresponsiveness (31,46,47). Similarly, studies in mice rendered eosinophil deficient by targeted disruption of the IL-5 gene (48) or in mice administered anti-IL-5 antibodies (14,45) demonstrated a role for IL-5-dependent eosinophilia in mediating airway hyperreactivity, whereas other studies using neutralizing antibodies could demonstrate no role for IL-5 or elevated tissue eosinophil numbers (8,49). These investigations have used different techniques for quantitating airway physiology, and they have used a variety of antigens for sensitization, some of which display genetic differences among various inbred strains of mice (18). It remains possible that multiple

pathways exist for the creation of allergic airways disease—one IL-4/$CD4^+$ T cell-dependent (8), one IL-5/eosinophil-dependent (14,48)—although, as shown here and elsewhere (8), we have been unable to define a necessary role for IL-5/eosinophils in mediating increases in lung resistance over a range of inbred mouse strains and allergens. In addition, by simultaneously assessing the importance of different immune cells and molecules in an optimized system, we have clearly demonstrated the greater importance of $CD4^+$ T cells, and IL-4, over other effector moieties.

The demonstration that $CD4^+$ T cells could mediate acute allergen-induced airways disease raises questions regarding the mechanism(s) involved. The mechanism is ultimately quite different than that mediated by immune complexes or antigen in Arthus-like reactions. Pulmonary inflammation was markedly reduced in mice deficient in either the substance P receptor, NK-1R, or the C5a anaphylatoxin receptor when challenged with antigen–antibody complexes (50), and cutaneous and systemic Arthus reactions were completely abrogated in mice with disruption of high- and low-affinity Fc receptors (51). The absence of immunoglobulin in the B cell–deficient mice or T cell–reconstituted RAG-deficient mice precluded activation of these types of inflammatory cascades. Potential interactions between Th2 cells and resident pulmonary cells that might serve as sources of distinct inflammatory pathways mediated by chemokines (11,52–56), interleukins (57), or the induction of homing and adhesion molecules (11,58) merit further study. Studies in B cell–deficient and RAG-reconstituted mice should be informative in defining the necessary and sufficient roles for such effector molecules. Although the requirement for IL-4 suggests that Th2 cells were required and sufficient to mediate disease, further studies will be needed to establish whether IL-4 production is necessary only from $CD4^+$ T cells, or also from endogenous cells of the innate immune system, or if IL-4 contributes directly or indirectly to the development of AH. It is noteworthy that these experiments could not define a necessary role for either $CD8^+$ T cells or $\gamma\delta$ T cells, although their possible regulatory contributions remain open.

An acknowledged caveat of these findings is their relevance to human asthma, a complex disease of unclear etiology that may encompass several different phenotypes. It is noteworthy that allergic diseases, including asthma, have been reported among rare individuals who completely lack eosinophils (59,60). Furthermore, an inverse relationship was noted between the predilection for asthma and the degree of $CD4^+$ T cell depletion among patients infected with HIV, despite the common association of elevated IgE levels with progressive disease (61). Thus, the association of elevated IgE and eosinophil levels with asthma may not indicate a causal role in the pathogenesis. In murine intestinal helminth infections, systems in which protective immunity is mediated by Th2 cells, no requisite role has been demonstrated for either IgE or eosinophils in immunity, despite their marked elevations in vivo (26,62).

The prevalence of asthma has risen steadily, such that 6–14% of children in the United States and Great Britain are affected (63,64). As noted above, a number of the pathologic and physiologic aspects of asthma are similar to those induced in this murine model. Human asthma is thought to be induced by a number of relatively common environmental antigens. Genetic studies in human populations have suggested linkage to areas near the cytokine locus that encodes IL-4, IL-5, IL-13, IL-9, and GM-CSF on chromosome 5q (65,66), raising the possibility that aberrant type-2 responses may occur in the lung because of genetic factors that concordantly link these cytokine responses. The inverse relationship demonstrated in population studies between the intensity of delayed type hypersensitivity, a type 1–mediated response, and the incidence of atopy and asthma, provides additional support for this hypothesis (67). The data reported here suggest that early interventions that target the activation and/or effector development of T cells specific for candidate environmental allergens might abrogate the subsequent development of lung disease by preventing the chronic recruitment of additional cells and mediators that amplify the tissue-destructive process. The model described should be useful for revealing the minimal interactions between $CD4^+$ T cells and endogenous cells of the lung that mediate airway pathology.

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