

Anti-IgE efficacy in murine asthma models is dependent on the method of allergen sensitization

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Background: Murine models used to delineate mechanisms and key mediators of asthma have yielded conflicting results and suggest that the dominant mechanism and mediators required for disease induction differ depending on the model and method of allergen sensitization used.

Objective: The goal of this study was to determine whether the mode of allergen sensitization influenced the role that IgE had in allergen-induced pulmonary eosinophilic inflammation.

Methods: Mice were exposed to dust mite extract in 2 models of allergic inflammation that differed in the method of sensitization. We compared sensitization by aerosol exposure with and without concomitant human respiratory syncytial virus infection with sensitization by means of systemic (intraperitoneal) exposure with adjuvant. After sensitization, animals were similarly challenged with aerosolized allergen. Animals were treated with anti-IgE mAb to deplete IgE and to determine its role in the induction of allergic inflammation and mucosa pathology in these models.

Results: Concomitant respiratory syncytial virus infection significantly enhanced allergen sensitization by aerosol exposure and exacerbated eosinophilic inflammation and airway mucosa pathology. Depletion of IgE in this model significantly reduced lung eosinophilic inflammation and airway mucosa pathology.

However, in the model in which animals were sensitized by means of systemic allergen exposure with adjuvant, depletion of IgE had no ameliorative effect on lung inflammation or pathology.

Conclusion: We demonstrated that the method of antigen sensitization can delineate the role of IgE in allergen-induced lung inflammation. In a murine model that more closely resembles ambient allergen exposure in human subjects, IgE had a critical role in the pathogenesis of allergic asthma and mucosa pathology. The results parallel the results reported with anti-IgE efficacy in allergic asthmatic human subjects. (*J Allergy Clin Immunol* 2001;107:1025-33.)

Key words: Respiratory syncytial virus, asthma, IgE, animal disease models, dust mite allergen

Eosinophil infiltration into the pulmonary parenchyma and airways is a hallmark of asthma, and the products of these cells are thought to be key to the generation of large-

Abbreviations used

BALF: Bronchoalveolar lavage fluid

PE: Phycoerythrin

RSV: Respiratory syncytial virus

airway pathology in asthma.¹⁻⁶ Clinical studies have also correlated clinical remission in asthmatic subjects with reduction or loss of eosinophil infiltration into the lung.⁷⁻⁹ The primary mechanisms contributing to airway eosinophil recruitment after allergen exposure in human asthmatic subjects are still unclear but are likely to include the activation of pulmonary CD4⁺ T lymphocytes and their secretion of cytokines and chemokines, as well as the resulting recruitment of effector cells, including both pulmonary mast cells and eosinophils.¹⁰ The role of IgE in contributing to mast cell degranulation and subsequent pulmonary eosinophilic inflammation has been inferred from numerous epidemiologic studies that demonstrated a relationship between levels of IgE and asthma.¹¹⁻¹⁶ Furthermore, there is now compelling evidence in human subjects that anti-IgE immunotherapy can improve the clinical course of allergic asthma.¹⁷⁻²⁰

A number of murine models of antigen-induced pulmonary eosinophilic inflammation have been developed to investigate the mechanisms underlying allergic airway disease, and the utility and limitations of these models have been reviewed.²¹⁻²⁴ Pulmonary eosinophilic inflammation and increased airway reactivity in these models can be suppressed by inhibiting a variety of mechanisms. These include elimination or suppression of T lymphocytes,²⁵⁻²⁸ elimination of T lymphocyte T_H2 cytokines with neutralizing antibodies or gene knock out,²⁹ inhibition of various chemokines,³⁰⁻³² inhibition of IgE,^{33,34} and inhibition of various adhesion molecules.³⁵⁻³⁹ The role of IgE has been evaluated in mice by using an anti-IgE antibody.^{33,34} Although a single administration significantly lowered circulating levels of IgE, the resulting inhibition of eosinophil migration into the bronchoalveolar lavage fluid (BALF) was short lived.³³

We evaluated the role of IgE in the induction of allergic pulmonary inflammation in murine models that differed in the method used to sensitize and thus induce an allergen-driven immune inflammatory response. Specifically, we evaluated aerosol sensitization as would occur in natural ambient exposure versus sensitization by means of systemic exposure with adjuvant. The latter is the most

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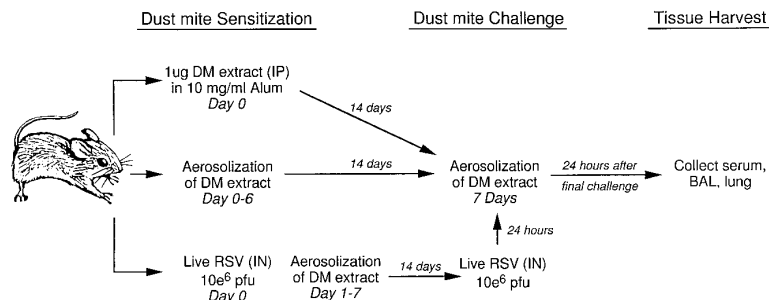


FIG 1. Schematic sensitization tools. Groups of C57/BL6 mice were sensitized to dust mite extract antigen by using the following 3 methods: (1) aerosolization alone; intraperitoneal inoculation with adjuvant (alum); and aerosolization with concomitant infection with human RSV. Two weeks after sensitization, the different groups of mice were then all similarly challenged with aerosol exposures of dust mite extract. *DM*, *D pteronyssinus*; *IN*, intranasal.

commonly used method reported. We chose an extract from the dust mite *Dermatophagoides pteronyssinus* as the allergen in each model because the immunodominant protein Der p 1 is a major human allergen.⁴⁰ In one model we further enhanced the allergic immune response to aerosolized dust mite allergen by coadministering live respiratory syncytial virus (RSV). RSV infection can exacerbate airway inflammation in asthmatic subjects⁴¹⁻⁴³ and is thought to be an important risk factor for the development of asthma in children.^{44,45} Furthermore, human RSV infection in mice can enhance airway sensitization to allergen and exacerbate the induced asthmatic phenotype in mice.⁴⁶ We then evaluated the efficacy of anti-IgE mAb therapy in these models, comparing sensitization by means of aerosol exposure to antigen with a more standard model in which sensitization was achieved by means of systemic exposure to allergen with adjuvant.

METHODS

Murine models of antigen sensitization and exposure

Pathogen-free 6- to 8-week-old female C57BL/6 mice were obtained from Charles River (Hollister, Calif) and were housed in static microisolators or high-efficiency particulate air filter-exhausted ventilated cages. The mice were given food and water ad libitum.

In the systemic sensitization model mice received primary immunization with 1 µg of standardized mite extract (*D pteronyssinus*; Bayer, Spokane, Wash) in 1 mg of alum (Intergen, Purchase, NY) by means of intraperitoneal injection on day 0. After 14 days, unanesthetized mice were rechallenged by means of aerosol allergen exposure with a 200 µg/mL solution of *D pteronyssinus* in endotoxin-free PBS (Hyclone, Logan, Utah) for 20 minutes each day for 7 consecutive days in a plexiglass chamber with an IS2 nebulizer (Pari Respiratory, Richmond, Va) driven by compressed air.

In the aerosol sensitization model mice received a primary immunization with the aerosol exposure protocol for *D pteronyssinus* allergen, as described. After 14 days, mice were rechallenged with aerosolized *D pteronyssinus* allergen daily for 7 consecutive days. The aerosol *D pteronyssinus* allergen sensitization protocol was further divided into 2 groups, in which one group was infected with 10⁶ pfu of live RSV instilled intranasally after achievement of anesthesia with isoflurane (Ohmeda Caribe, Liberty Corner, NJ). Beginning 1 day after RSV infection, mice were sensitized with *D pteronyssinus* antigen by means of the aerosol exposure protocol.

After 14 days, mice were reinfected with RSV and rechallenged with aerosolized *D pteronyssinus*, as previously described.

Anti-IgE treatment

In the intraperitoneal *D pteronyssinus* sensitization protocol mice were treated with 100 µg of either a monoclonal rat anti-mouse IgE or isotype control antibody 1 day before and the day of *D pteronyssinus* sensitization. At the time of aerosol *D pteronyssinus* rechallenge, mice were again treated intraperitoneally with antibody and continued to receive antibody during each day of *D pteronyssinus* aerosol exposure. In the aerosol *D pteronyssinus* sensitization model mice were injected intraperitoneally with 100 µg of antibody 2 days before and the first day of aerosol allergen exposure during sensitization. Anti-IgE antibody treatment was repeated as described above at the time of *D pteronyssinus* aerosol rechallenge, and thus for each model, all groups received equivalent anti-IgE dosing.

Reagents

RSV. RSV strain A2 (ATCC, Rockville, Md) was maintained in Hep-2 cells (ATCC) maintained in Eagle's minimal essential medium supplemented with glutamine, amphotericin, gentamicin, penicillin G (Gibco BRL, Grand Island, NY), and 10% FBS (Hyclone, Logan, Utah). Viral stocks were prepared as described previously^{47,48} at a titer of 10⁷ pfu/mL.

Antibodies. A rat IgG1 anti-IgE (R35-92), rat IgG1 isotype control, FITC-conjugated anti-mouse CD23, phycoerythrin (PE)-conjugated anti-B220, and FITC- and PE-conjugated isotype control antibodies were obtained from Pharmingen (San Diego, Calif).

ELISA

Total mouse IgE was measured with ELISA, as previously described.⁴⁹ The detection level was 1.56 ng/mL. *D pteronyssinus*-specific IgG1 was measured by using a sandwich ELISA with a *D pteronyssinus* coat concentration of 20 µg/mL in 0.05 mol/L carbonate buffer (pH 9.6) and by detecting IgG1 binding with horseradish peroxidase-conjugated goat anti-mouse IgG1 (Caltag, South San Francisco, Calif) at a 1:5000 dilution. The detection level was 30 pg/mL. Murine IL-5 was measured by using an ELISA according to the manufacturer's protocol (Pharmingen), with a detection level of 30 pg/mL.

Tissue harvest and BALF analysis

Serum was collected from the mice by means of retro-orbital puncture 24 hours after the final aerosol challenge. Immediately after euthanasia of animals, BALF was collected by means of tracheal cannulation with 3 repeated lavages with 0.5 mL of HBSS

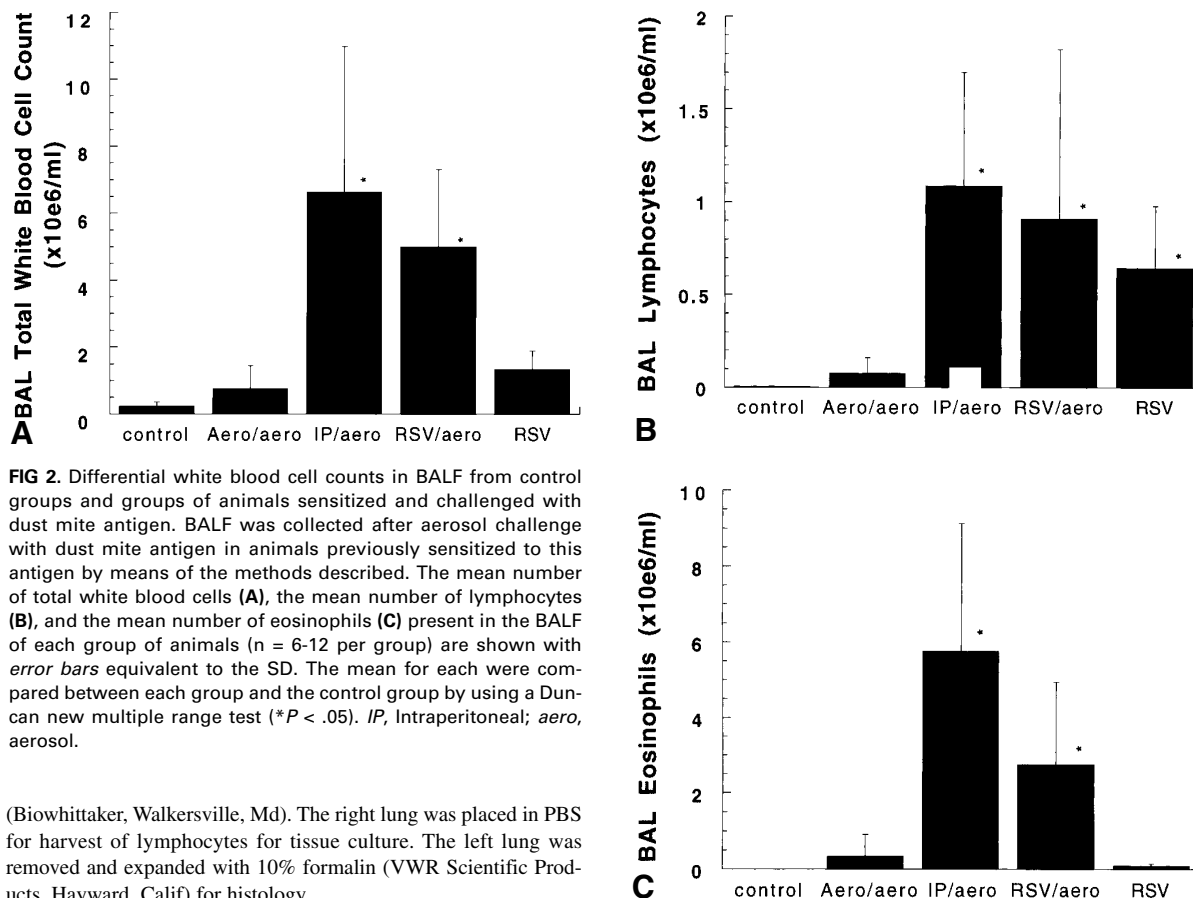


FIG 2. Differential white blood cell counts in BALF from control groups and groups of animals sensitized and challenged with dust mite antigen. BALF was collected after aerosol challenge with dust mite antigen in animals previously sensitized to this antigen by means of the methods described. The mean number of total white blood cells (**A**), the mean number of lymphocytes (**B**), and the mean number of eosinophils (**C**) present in the BALF of each group of animals ($n = 6$ –12 per group) are shown with error bars equivalent to the SD. The mean for each were compared between each group and the control group by using a Duncan new multiple range test (* $P < .05$). *IP*, Intraperitoneal; *aero*, aerosol.

(Biowhittaker, Walkersville, Md). The right lung was placed in PBS for harvest of lymphocytes for tissue culture. The left lung was removed and expanded with 10% formalin (VWR Scientific Products, Hayward, Calif) for histology.

BALF samples were analyzed for complete blood counts on an H1E analyzer (Bayer Technicon, Tarrytown, NY). Differential cell analyses were performed manually by staining cytospin preparations with DiffQuik (Dade, Aguanda, Puerto Rico). The induction of IL-5 production by concanavalin A treatment of single-cell suspensions of pulmonary lymphocytes was measured by first dispersing lung tissue with glass slides, washing and counting cells, and then culturing 5×10^5 cells/mL at 37°C in RPMI medium supplemented with glutamine, penicillin-streptomycin, nonessential amino acids, pyruvate (Gibco BRL), FBS (Hyclone), and 2 µg/mL concanavalin A (Sigma, St Louis, Mo) for 72 hours. IL-5 in the cell culture supernatants was measured by means of ELISA on day 2.

Splenocyte CD23 expression

Splenocytes from individual mice were isolated from minced whole spleen cells, and contaminating red blood cells were lysed with a standard lysis buffer (NH_4Cl , KHCO_3 , tetrasodium EDTA). Lymphocytes resuspended in PBS containing 0.1% BSA and 0.01% sodium azide were stained with FITC anti-CD23, PE anti-B220, or conjugated control IgG for 30 minutes at 4°C. Cells from all groups were washed twice and then analyzed on a flow cytometer FACScan (Becton Dickinson, Mountain View, Calif). Both forward- and side-scatter gates were used to acquire lymphocytes by using Cell Quest software (Becton Dickinson, San Jose, Calif). The mean fluorescent intensity (MFI) of FITC CD23-staining, PE B220-gated lymphocytes was analyzed for CD23 expression.

Histology

Lungs were fixed in 10% neutral-buffered formalin and embedded in paraffin, and 3-µm thick sections were stained with hematoxylin and eosin or Alcian blue-periodic acid-Schiff.⁵⁰ Lung

inflammation was scored on a 0- to 4-point scale (minimal, mild, moderate, and severe, respectively) on the basis of the severity of eosinophilic and lymphocytic inflammatory cell infiltrates. The thickness of the mucosa of the main intrapulmonary lobar bronchi was determined by measuring the distance from the basal lamina to the luminal surface with a micrometer at 600× magnification. A mean value for each animal was calculated from 6 measurements for each main lobar bronchus evaluated. The mean mucosa thickness for each group was then determined.

RESULTS

Allergen sensitization and airway inflammation

Mice were sensitized to dust mite allergen by means of one of the 3 following methods: (1) systemic exposure by intraperitoneal administration of the allergen in adjuvant; (2) pulmonary airway exposure by aerosol administration of the allergen alone; or (3) pulmonary airway exposure by aerosol administration of the allergen with concurrent respiratory infection with human RSV (Fig 1). After sensitization, mice were rechallenged with dust mite allergen by means of daily aerosol exposure for 7 days. Groups were then compared by evaluation of BALF differential cell counts, serum and BALF IgE, serum dust mite antigen-specific IgG1, splenocyte B-lymphocyte CD23 expression, pulmonary histology, and pulmonary IL-5 secretion.

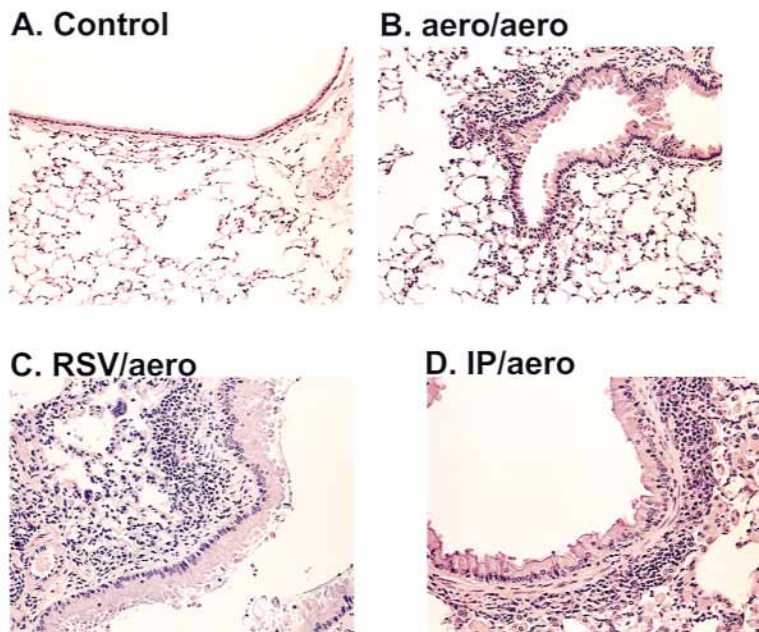


FIG 3. Histopathology. Representative 3- μ m thick sections of formalin-fixed, paraffin-embedded lung specimens stained with hematoxylin and eosin are shown from animals in the control group (A), the aerosol/aerosol (*aero/aero*) group (B), the RSV/aerosol (*RSV/aero*) group (C), and the intraperitoneal/aerosol (*IP/aero*) group (D). Lung specimens were evaluated histologically to assess the degree of inflammation and changes in the mucosa epithelium. There was consistent, significant, moderate eosinophilic and lymphocytic inflammation and marked mucosa epithelial cell hypertrophy with increased mucus production in animals in the intraperitoneal/aerosol and the RSV/aerosol groups. Inflammation and mucosa changes in the lung specimens of the aerosol/aerosol group animals were present but were less severe. In control animals there was no inflammation and no mucosa hypertrophy. In each group 6 to 12 animals were evaluated.

Each model of allergen sensitization and challenge resulted in inflammation of different severities. Sensitization by means of systemic intraperitoneal exposure followed by aerosol challenges (intraperitoneal/aerosol group) or sensitization by means of primary aerosol exposure with concurrent RSV infection followed by aerosol challenges (RSV/aerosol group) induced a consistent similar marked increase in BALF eosinophils and lymphocyte counts ($P < .05$, Fig 2). Infection with human RSV only was done as a control and induced a minimal increase in the numbers of lymphocytes and alveolar macrophages in the BALF but produced no eosinophilic inflammation (Fig 2).^{47,48} Sensitization by means of primary aerosol exposure alone followed by aerosol challenges (aerosol/aerosol group) induced significant but less severe inflammation than that seen in the other 2 models (Fig 2). The 5-fold increase in BALF eosinophils in the RSV/aerosol group compared with that in the aerosol/aerosol group demonstrates that, as others have reported,⁴⁶ RSV infection can markedly exacerbate pulmonary allergic inflammation in response to aerosolized allergen.

The differences observed in the magnitude of BALF inflammatory cell infiltrates and pulmonary inflammation was confirmed with pulmonary histology. Animals in the intraperitoneal/aerosol and RSV/aerosol groups had equivalent moderate-to-severe perivascular and peribronchiolar eosinophil and lymphocyte cell infiltrates and equivalent significant mucosa thickening in the pri-

mary lobar bronchi (Fig 3). In the aerosol/aerosol group the pattern of inflammation was again similar, but the severity of inflammation and mucosa thickening was significantly less (Fig 3).

Total serum IgE levels were markedly elevated after secondary aerosol allergen challenge in the intraperitoneal/aerosol group and in the RSV/aerosol group compared with levels in either the aerosol/aerosol group or control animals (Table I). IgE levels in BALF were greatest in the RSV/aerosol group. The marked increase in serum and BALF IgE in the RSV/aerosol group compared with that in the aerosol/aerosol group correlated with the observed enhanced inflammatory response. Interestingly, dust mite-specific IgG1 titer was greatest in the intraperitoneal/aerosol group, in which primary sensitization occurred by means of systemic immunization with adjuvant. Primary sensitization by means of pulmonary mucosa exposure to aerosolized dust mite allergen alone or in combination with RSV infection resulted in a relatively poor IgG1 response (Table I).

In our evaluation pulmonary lymphocyte cytokine production 24 hours after the final aerosol challenge with dust mite allergen and levels of IFN- γ , IL-4, and IL-2 released from stimulated lung lymphocytes were below the detection limit of the assay (data not shown). IL-5 release, however, was detectable in all of the experimental groups but was greatest in the intraperitoneal/aerosol and RSV/aerosol groups (Table I).

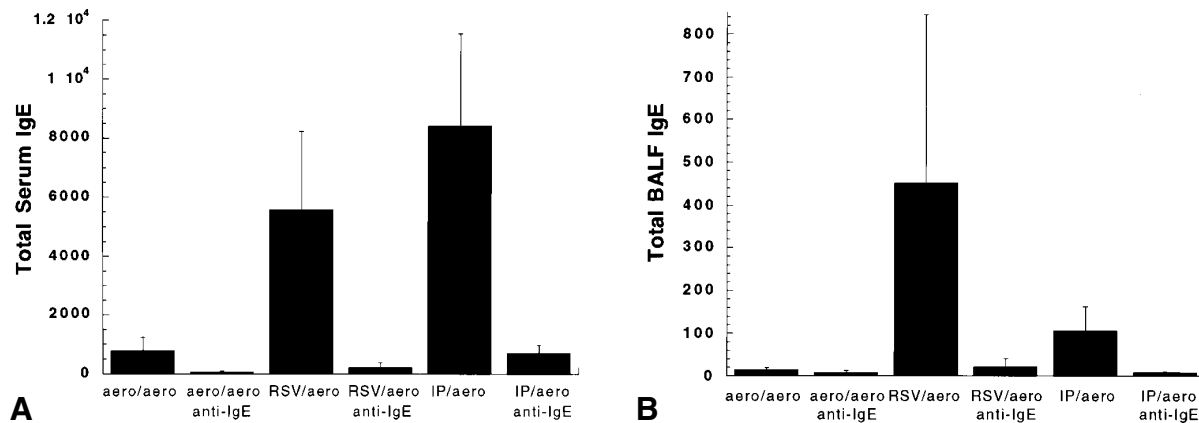


FIG 4. The effect of anti-IgE antibody on mean total serum and BALF IgE levels. Intraperitoneal/aerosol (IP/aero) and RSV/aerosol (RSV/aero) group animals sensitized to dust mite antigen by means of the methods described were administered anti-IgE antibody or were left untreated ($n = 6$ per group) just before and during challenged with aerosolized dust mite antigen. The total IgE levels from serum and BALF were measured by using ELISA. Anti-IgE treatment significantly reduced the IgE levels in the serum and BALF in all groups of animals evaluated, as determined by using a Duncan new multiple range test ($P < .05$).

TABLE I. Mean IgE, IgG1, and IL-5 levels by group

Group	Total IgE (ng/mL)		<i>D pteronyssinus</i> -specific IgG1 in serum	IL-5 in supernatant (pg/mL)
	Serum	BALF		
Control	182 ± 56	0.40 ± 0.41	BDL	BDL
Aerosol/aerosol	431 ± 434	10.2 ± 13.8	32 ± 0	23 ± 37
Intraperitoneal/aerosol	4959 ± 3520*	111.9 ± 101.6	4044 ± 3624	1244 ± 756
RSV/aerosol	5338 ± 2023*	287.8 ± 286.2*	922 ± 908	710 ± 389
RSV	0.88 ± 0.12	21.5 ± 4.7	ND	ND

The mean concentration of IgE and the mean dust mite-specific serum IgG1 titer was determined by using ELISA and compared with that of the control group by using a Duncan new multiple range test. IL-5 was assayed by using ELISA in supernatants of cultures of concanavalin A-stimulated lymphocytes isolated from digests of lungs. In the control group IL-5 concentration was less than the standard and thus was below the detectable limits of the assay. The mean IL-5 levels for each group were compared with that of the control group by using a Duncan new multiple range test.

BDL, Below detectable limits; ND, not done.

* $P < .05$.

Anti-IgE treatment

We evaluated the effect of IgE depletion in the 3 models described above to determine whether the method of allergen sensitization influences the role IgE plays in eosinophilic allergic pulmonary inflammation. In all animals administration of the anti-IgE antibody resulted in a striking decrease in serum and BALF IgE levels and a 10-fold reduction in the intraperitoneal/aerosol and RSV/aerosol groups (Fig 4). Because it has been shown that IgE levels affect the expression of CD23, the low-affinity IgE receptor on B lymphocytes in the mouse,⁵⁰ we evaluated CD23 expression in B lymphocytes by means of flow cytometry. In the intraperitoneal/aerosol group the MFI was reduced by 51%, and in RSV/aerosol group animals anti-IgE treatment was reduced the MFI by 39%.

Although total IgE was reduced to equivalent low levels in all animals treated with the anti-IgE mAb, the response to IgE depletion was strikingly different between the groups. BALF eosinophil cell counts and lung pathology were only significantly reduced in the 2 models that used

aerosol exposure during both sensitization and rechallenge: the aerosol/aerosol and the RSV/aerosol groups. There was no effect of IgE depletion on pulmonary inflammation in the intraperitoneal/aerosol group (Fig 5). In the RSV/aerosol group anti-IgE treatment reduced the mean number of BALF eosinophils by a factor of 10 compared with that seen in untreated animals in the group. The anti-IgE-treated animals in the aerosol/aerosol and RSV/aerosol groups also exhibited a consistent and parallel reduction in the numbers of perivascular and peribronchiolar eosinophils, as determined by histology. Interestingly, although the infiltration of eosinophils in the lung was markedly inhibited by treatment with anti-IgE mAb in the RSV/aerosol group, there was no significant effect on the infiltration of lymphocytes into the lung compared with that seen in untreated animals (Fig 5, B).

Eosinophils have been postulated to have a significant role in the pathogenesis of large-airway pathology in human asthma by mediating both mucosa epithelial damage and causing damage to the underlying peribronchial smooth muscle and stroma.^{3,4,51} Both the intraperi-

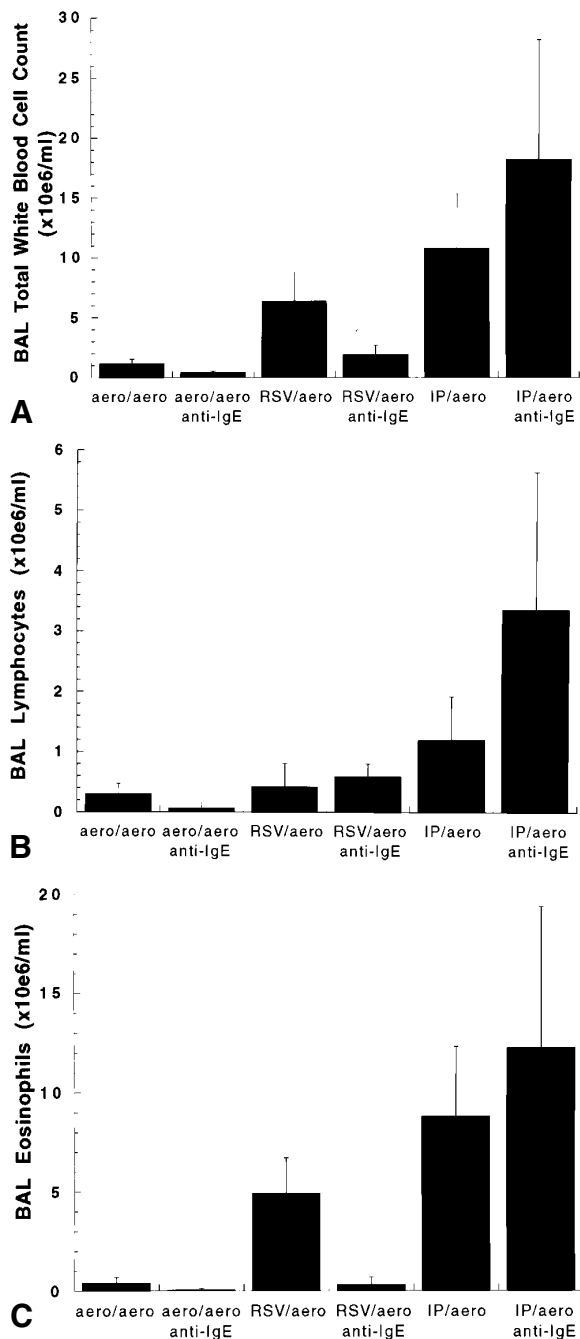


FIG 5. The effect of treatment with anti-IgE antibody on the differential white blood cell counts in BALF. Groups of animals were treated with anti-IgE antibody or were left untreated ($n = 6$ per group). BALF was collected after aerosol challenges with dust mite antigen in animals previously sensitized to allergen by means of the methods described. The mean number of total white blood cells (**A**), the mean number of lymphocytes (**B**), and the mean number of eosinophils (**C**) present in the BALF of each group of animals are shown with error bars equivalent to the SD. The means were compared between anti-IgE-treated and untreated groups by using a Duncan new multiple range test ($P < .05$). Anti-IgE treatment significantly reduced eosinophil infiltration into the lungs of animals in the aerosol/aerosol (*aero/aero*) and the RSV/aerosol (*RSV/aero*) groups ($P < .05$) but had no effect on the number of BALF eosinophils in animals in the intraperitoneal/aerosol (*IP/aero*) group.

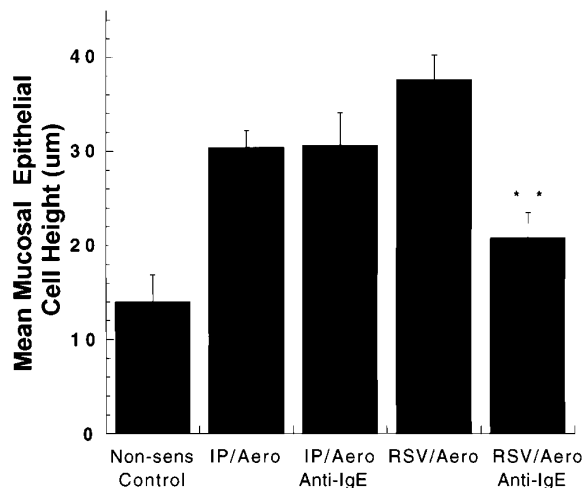


FIG 6. The effect of anti-IgE treatment on mucosa epithelial cell hypertrophy. The average thickness of the mucosa epithelium of the main intrapulmonary lobar bronchi was determined for normal animals, untreated animals in the intraperitoneal/aerosol (*IP/aero*) group, anti-IgE-treated animals in the intraperitoneal/aerosol group, untreated animals in the RSV/aerosol (*RSV/aero*) group, and anti-IgE-treated animals in the RSV/aerosol group ($n = 6$ per group). For each animal in each group, a mean thickness of the mucosa was determined by measuring the height of the mucosa from the basement membrane to the luminal surface of the main intrapulmonary lobar bronchi in 3-μm thick hematoxylin and eosin-stained sections of formalin-fixed, paraffin-embedded lung tissue. Anti-IgE treatment resulted in significant abrogation of mucosa thickening and mucosa epithelial cell hypertrophy in the RSV/aerosol group animals but had no effect on the mucosa height in intraperitoneal/aerosol group animals compared, as compared by using a Duncan new multiple range test ($*P < .05$).

toneal/aerosol and RSV/aerosol models produced a similar degree of mucosa thickening (Fig 6). IgE depletion by means of anti-IgE treatment, however, only had a significant effect on mucosa thickening in the RSV/aerosol model, in which the mean thickness of the mucosa in the lobar bronchi in IgE-depleted animals was $21 \pm 2.7 \mu\text{m}$ compared with $38 \pm 2.6 \mu\text{m}$ in untreated animals ($P < .05$). Naive control animals that had no allergen sensitization had a mean mucosa height in the main lobar bronchi of $14 \pm 2.9 \mu\text{m}$. IgE depletion in the RSV/aerosol group also resulted in a reduction in mucin production by the mucosa epithelium (Fig 7). In striking contrast, IgE depletion had no effect on mucosa thickening or mucin production in animals in the intraperitoneal/aerosol group (Fig 7, *G* and *H*).

DISCUSSION

In murine models of human asthma, it is apparent that initiation of the allergic immune response is T lymphocyte dependent.^{25,26} However, the predominant direct mediator of allergic eosinophilic inflammation, including eotaxin and other chemokines,³⁰⁻³² murine IgG1^{52,53} or IgE,^{33,34} or T-lymphocyte cytokines (eg, IL-5),²⁹ appears to vary experimentally and may be dependent on the

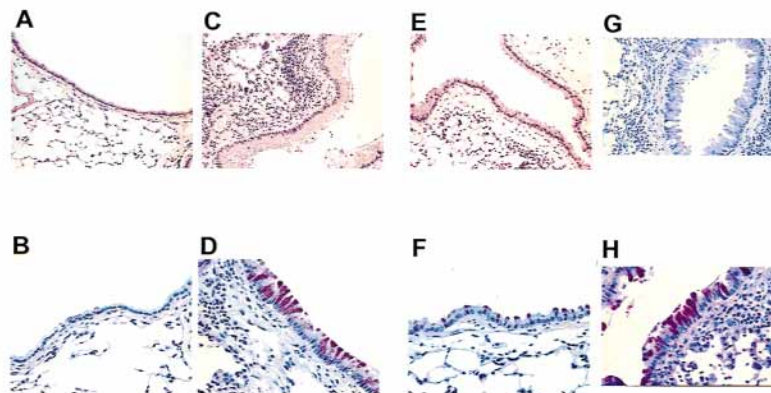


FIG 7. The effect of anti-IgE treatment on mucosa pathology in RSV/aerosol group animals. Representative histologic sections of main intrapulmonary lobar bronchi stained with routine hematoxylin and eosin or with Alcian blue-PAS dye are shown from a control animal (**A** and **B**), an untreated animal in the RSV/aerosol group (**C** and **D**), an anti-IgE-treated animal from the RSV/aerosol group (**E** and **F**), and an anti-IgE-treated animal from the intraperitoneal/aerosol group (**G** and **H**). The hematoxylin and eosin-stained sections (**A**, **C**, **E**, and **G**; original magnification 400 $\times$) demonstrate the ameliorative effect of anti-IgE treatment in this model on both inhibiting eosinophilic inflammation and on decreasing mucosa epithelial cell hypertrophy. The Alcian blue-PAS stained sections (**B**, **D**, **F**, and **H**; original magnification 800 $\times$) demonstrate the relative amount of mucin (stained bright red) present in the lobar bronchial mucosa. The height and relative amount of mucin in the mucosa epithelial cells of the lobar bronchi were reduced with anti-IgE treatment in the RSV/aerosol group animals. Anti-IgE treatment, however, had no effect on inflammation, mucosa height, or relative mucin production in intraperitoneal/aerosol group animals.

model, strain of mouse, or allergen used.^{22,54-56} Delineating the mediators of allergic asthma in animal models is important but, arguably, is useful only if one understands the relevance of such studies to the pathogenesis of human asthma.

Here we mimicked natural ambient exposure of allergen by sensitizing and challenging mice with dust mite allergen delivered only through the aerosol route. In previous studies we used aerosolized ovalbumin and found it to be poorly allergenic, even when aerosolized multiple times at 2- to 3-week intervals for up to 6 months (unpublished observation). Our observation is likely not unique, as evidenced by the fact that most studies that use ovalbumin as an allergen typically use systemic exposure in combination with a potent adjuvant to achieve sensitization.⁵⁷ In our study aerosolization of dust mite allergen was capable of inducing allergic pulmonary inflammation that could be inhibited with anti-IgE treatment. A possible explanation for the enhanced allergenicity of Der p 1 dust mite extract over ovalbumin is the reported ability of Der p 1 dust mite extract to enzymatically cleave cell-surface B-lymphocyte CD23 and T-lymphocyte CD25,^{58,59} resulting in subsequent dysregulation of IgE synthesis and exacerbation of a T_H2-type T-lymphocyte response.^{40,60}

In this study, as has been reported by others,⁴⁶ we have clearly demonstrated that concurrent RSV infection in mice exacerbates the development of allergic asthma, including pulmonary leukocytic infiltration, production of IgE and IL-5, and induction of large-airway mucosa pathology. RSV infection in mice can induce a variety of proinflammatory cytokines, enhance T-cell responsiveness, induce the upregulation of intercellular adhesion molecule 1 on respiratory epithelial cells, enhance airway sensitization to allergen, and exacerbate human

asthma.^{46,61-63} RSV infection in human subjects is both a risk factor for the development of asthma and can exacerbate asthma symptoms in adults and children.⁴¹⁻⁴⁵ This model allowed us to induce consistent moderate-to-severe pulmonary eosinophilic inflammation, in which sensitization occurred entirely by means of airway exposure and was done without the use of systemic sensitization with adjuvant.

Our results clearly demonstrate that pulmonary eosinophilic inflammation and mucosa pathology can be inhibited by anti-IgE and thus can be driven by IgE. Interestingly, this was the case in the models that used aerosolization as the only route of exposure to allergen, but there was no apparent dependence on IgE in the more standard intraperitoneal/aerosol model. The basis for this difference, and possibly the conflicting reports in the literature about IgE in murine asthma, may lie in the fact that although in human subjects IgE is the only antibody capable of causing mast cell degranulation, in mice both IgE and IgG1 can bind to and cause degranulation of mast cells and basophils. Murine mast cells and basophils not only express Fc ϵ RI as their human counterpart but also uniquely express Fc-Fc γ RIIb1, Fc γ RIIb2, and Fc γ RIII Ig receptors. Murine mast cells and basophils can thus bind to and can be activated by both IgE and IgG.^{52,53,64,65} This significant and important difference between human subjects and rodents is important because it enables rodent mast cells to have an active role in allergic asthma models in the absence of IgE. Oshiba et al⁵³ have clearly shown this by demonstrating that passive transfer of ovalbumin-specific IgG1 can induce allergic asthmatic pulmonary disease in mice with subsequent exposure to aerosolized allergen. It is thus critical to consider both the role of allergen-specific IgE and IgG in mice to accurately

ly interpret data and to assess the correlation between IgE and experimentally induced allergic asthma in mice. This may have the greatest significance in murine models that use systemic immunization with an adjuvant that markedly enhances T_H2 responses and thus the production of high levels of both IgG1 and IgE antibodies. The lack of efficacy of anti-IgE therapy in the systemic sensitization model (intraperitoneal/aerosol) suggests that other mediators were induced, including IgG1, that could drive allergic pulmonary eosinophilic inflammation.

Our findings have important implications for interpreting the results of preclinical animal models of asthma and for helping to clarify the conflicting evidence on the role of IgE in murine asthma models. Furthermore, our findings suggest that the responsiveness to anti-IgE therapy in human subjects may similarly depend on the relative dominance of IgE versus other possibly redundant mediators that can also cause asthmatic disease in human subjects. Further preclinical evaluation of the therapeutic use of anti-IgE mAbs, novel small-molecule antagonists of IgE, or other therapeutic strategies to inhibit IgE synthesis will be greatly improved by the availability of an IgE-dependent murine model of allergic pulmonary eosinophilic inflammation, such as the model described here.

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