Dissociation of Airway Hyperresponsiveness from Immunoglobulin E and Airway Eosinophilia in a Murine Model of Allergic Asthma

Julie A. Wilder, D. David S. Collie,* Bridget S. Wilson, David E. Bice, C. Richard Lyons, and Mary F. Lipscomb

Departments of Pathology and Internal Medicine, University of New Mexico; and New Mexico and Lovelace Respiratory Research Institute, Albuquerque, New Mexico

Nonspecific airway hyperresponsiveness (AHR) is a hallmark of human asthma. Both airway eosinophilia and high serum levels of total and antigen-specific immunoglobulin E (IgE) are associated with AHR. It is unclear, however, whether either eosinophilia or increased IgE levels contribute directly to, or predict, the development of AHR. Investigations conducted with various murine models of asthma and different mouse strains have resulted in conflicting evidence about the roles that IgE and airway eosinophilia play in the manifestation of AHR. We show that systemic priming with ovalbumin (OVA) in alum, followed by a single day of OVA aerosol challenge, is sufficient to induce AHR, as measured by increased pulmonary resistance in response to intravenously delivered methacholine in BALB/c, but not C57BL/6 or B6D2F1, mice. This was observed despite the fact that OVA-challenged BALB/c mice had less airway eosinophilia and smaller increases in total IgE than either C57BL/6 or B6D2F1 mice, and had less pulmonary inflammation and OVA-specific IgE than B6D2F1 mice. We conclude that airway eosinophilia, pulmonary inflammation, and high serum levels of total or OVA-specific IgE are all insufficient to induce AHR in C57BL/6 and B6D2F1 mice, whereas BALB/c mice demonstrate AHR in the absence of airway eosinophilia. These data confirm that the development of AHR is genetically determined, not only in naive mice, but also in actively immunized ones, and cannot be predicted by levels of airway eosinophilia, pulmonary inflammation, total IgE, or antigen-specific IgE.

bronchoconstrictors in both the naive (11) and immune states (6, 12), with BALB/c mice being more responsive than C57BL/6 mice. We included B6D2F1 mice as an essential control in our analysis, to ensure that we could confirm the results initially reported by Kung and colleagues, in which airway eosinophilia was substantial (10), and extended their observations by including quantitative measures of pulmonary inflammation, ovalbumin (OVA)-specific immunoglobulin levels, and AHR. Our results show that after systemic priming with OVA in alum on two occasions 5 d apart, and two OVA aerosol challenges given on a single day 1 wk after the last systemic dose of OVA, only BALB/c mice exhibited AHR in response to intravenously delivered methacholine. This was surprising because BALB/c mice had less airway eosinophilia and smaller increases in total serum IgE than either C57BL/6 or B6D2F1 mice, and less pulmonary inflammation and OVA-specific IgE than B6D2F1 mice after OVA aerosol exposure. Our data therefore suggest that none of these parameters can directly lead to or predict the development of nonspecific AHR in the mouse model of allergic asthma. Rather, our data support the hypothesis that nonspecific AHR is determined by the genetic background of the mouse in both the naive state, as has been previously reported (11), and during episodes of antigen-driven pulmonary inflammation and increased serum IgE levels.

Materials and Methods

Mice

BALB/c mice were bred at the University of New Mexico Animal Resources Facility (UNM ARF). C57BL/6 mice were either bred at this same facility or purchased from Jackson Laboratories (Bar Harbor, ME). B6D2F1 (C57BL/6 x DBA/2 F1) mice were purchased from Jackson Laboratories. All mice were housed under specific-pathogen-free conditions and used between 6 and 14 wk of age. All animal protocols were reviewed and approved by the UNM ARF.

Sensitization and Challenge with OVA

Mice were sensitized with OVA (Grade V, Sigma Chemical Co., St. Louis, MO) adsorbed to Al(OH)3 gel (A Idrich Chemical Company, Milwaukee, WI), using 8 mg OVA/2 ml gel/0.5 ml H2O, pH 7.0–7.5, given intraperitoneally on Days 0 and 5 of each experiment. On Day 12, mice were placed in a nose-only aerosolization chamber (Intox, Albuquerque, NM) and exposed to 0.5% OVA in pH-neutural saline, or to saline alone, which was nebulized with a Lovelace Nebulizer (Intox) for a period of 1 h in the morning and 1 h in the afternoon. The aerosol particle size was less than 1 μm (6 liters air/min; 42 psi).

Harvest of Tissue and Fluid

All mice were killed by inhalation of CO2 on Day 15 of the experiment. Serum was collected by severing the renal artery. Tracheobronchial lavage (TBL) was performed with a single instillation and removal of 0.3 ml phosphate-buffered saline (PBS) containing 0.6 mM ethylenediaminetetraacetic acid through PE-150 tubing inserted into a small incision in the trachea. Cells recovered from the TBL were counted and used for cytospin preparations (25,000 cells or less per slide). These cytospins were stained with the Baxter Diff-Quik kit (VWR Scientific Products, San Francisco, CA), and differentials were calculated. In some animals, 10% buffered neutral formalin fixative was injected through the syringe to inflate the lungs. The lungs, once excised, were fixed for at least 24 h before the left lobes were sectioned longitudinally along the major airway, and were submitted to the UNM Hospital Pathology Laboratory (Albuquerque, NM), where they were embedded, sectioned, and stained with hematoxylin and eosin (H&E).

Immunoglobulin Enzyme-Linked Immunosorbent Assays

OVA-specific IgG, in the mouse sera was measured with OVA-coated polystyrene microtiter (PVC) plates (Falcon; Fisher Scientific, Pittsburgh, PA; 0.1 mg/ml OVA). Plates were coated overnight with OVA in PBS at 4°C, after which they were washed with double distilled H2O (ddH2O) and blocked with 5% (wt/vol) nonfat milk. Sera were diluted in PBS containing 0.25% bovine serum albumin and 0.05% Tween 20 (blocking buffer). Three dilutions of sera per mouse were added to the plates in duplicate, and incubation was done overnight at 4°C. The plates were then washed with ddH2O and incubated for 10 min at room temperature (RT) with blocking buffer in the wells, followed by washing in ddH2O. OVA-specific IgG was detected using horseradish peroxidase (HRP)-coupled conjugated goat antimouse IgG (Southern Biotechnologies, Birmingham, AL) diluted in blocking buffer, with incubation for 2 h at RT. A fter washing as described previously, HRP substrate (2,2’-azino-bis-[3-ethylbenzothiazoline]-6-sulfonic acid; Sigma) was added and color development proceeded at RT until the least dilute serum sample approached an OD of 1.0–2.0. At this point, the plates were read on a Dynatech enzyme-linked immunosorbent assay (ELISA) reader (Bio-Tek Instruments Inc., Winooski, VT). The OD of each serum dilution was multiplied by its dilution factor to give an arbitrary unit. For each mouse, the units of anti-OVA IgG1 are calculated from the three serum dilutions, and an average is presented. Total IgE levels in the serum were determined with a sandwich ELISA, using PVC plates coated with rat antimouse IgE (Clone R 35; Pharmingen, San Diego, CA; 2 μg/ml) and rat antimouse IgE–HRP (Southern Biotechnologies). Coating, serum dilution, washing, and development procedures were done as described for the OVA-specific IgG1 ELISA. A known amount of monoclonal IgE antibody was used to construct a standard curve in each assay, and the values are reported as ng/ml.

OVA-Specific IgE Bioassay

RBL-2H3 cells, a rat basophilic leukemia cell line, were loaded with [3H]-serotonin before being incubated in duplicate with 5%, 2.5%, or 1.25% normal mouse serum or serum from OVA-immune mice. In the OVA-specific bioassay for IgE, IgE present in the sera binds to the high affinity FcεRI expressed by the RBL-2H3 cells. Either OVA (5 μg/ml) or anti-IgE antibody (1 μg/ml; used as a positive control) was added to crosslink the bound IgE. The release of [3H]-serotonin from the cell granules was
were ventilated at a rate of 150 breaths/min and a V_t cannula was fitted to the male connector and the mice adhesive. Once placed in the plethysmograph, the tracheal inserted and were sealed and secured with cyanoacrylate saline) and a tracheal catheter (20-gauge needle hub) were to a 30-gauge needle and filled initially with heparinized saline. Both a tail-vein catheter (10 cm of PE-10 tubing attached of a four-way connector protruded into the box containing the screen pneumotachograph was 0.322 Pa/ml/s. One port of a four-way connector protruded into the box containing the anesthetized animal and served as the male connector for the tracheal cannula. Two of the other ports were connected to the inspiratory and expiratory lines of a modified rodent ventilator (Model 683; Harvard Apparatus, South Natick, MA), and the last port was connected to a second differential pressure transducer, the other side of which was connected to the plethysmograph chamber. Mice were anesthetized by intraperitoneal injection of a 2; 25–50% involvement = 3; and > 50% involvement = 4. The scores of each of the three sections were averaged to obtain a peribronchiolar, periarterial, and perivenular score for each lung. The total lung score represents the sum of the scores of these three areas. Two independent observers (J. A. W. and M. F. L.) scored each lung lobe in a blinded fashion. The average of the two observations is reported for each mouse examined. Data presented are the average (± SE M) of individual mice from several experiments.

Lung Histology
Lungs were excised, sectioned, and stained with H & E as described earlier. Three 10× fields per lung were examined and scored separately for the percent inflammatory cell infiltration around bronchioles, peribronchial arteries, and veins, according to the following scheme: less than 1% of the circumferential or longitudinal area involved with any type of inflammation was scored as 0; 1–5% involvement = 0.5; 5–10% involvement = 1; 10–25% involvement = 2; 25–50% involvement = 3; and > 50% involvement = 4. Between-group dose–response curves showing percent degranulation of [3H]-serotonin–loaded RBL-2H3 cells, as a measure of OVA-specific IgE, were compared through the use of ANOVA statistics. Differences in all other measured variables were analyzed either with one-way or two-way ANOVA statistics followed by the Bonferroni/Dunn post hoc test to examine all possible pairwise comparisons. Values of P < 0.05 were considered significant for all comparisons.

Statistics
Differences between groups in R_L responses to methacholine were analyzed with a general linear model analysis of variance (ANOVA) after rank transformation of the data. Between-group R_L responses at individual doses of methacholine were compared with unpaired t tests to determine significance. Between-group dose–response curves showing percent degranulation of [3H]-serotonin–loaded RBL-2H3 cells, as a measure of OVA-specific IgE, were compared through the use of ANOVA statistics. Differences in all other measured variables were analyzed either with one-way or two-way ANOVA statistics followed by the Bonferroni/Dunn post hoc test to examine all possible pairwise comparisons. Values of P < 0.05 were considered significant for all comparisons.

Results
AHR
BALB/c, C57BL/6, and B6D2F1 mice, previously immunized intraperitoneally with OVA in alum, received aerosols of either saline or OVA for 60 min twice within a single day. Three days later, anesthetized, mechanically ventilated mice were studied in a whole-body volume-displacement plethysmograph, and R_L was measured (Figure 1). OVA–challenged BALB/c, but not C57BL/6 or B6D2F1 mice, exhibited significantly increased R_L in response to increasing doses of intravenously administered methacholine, as compared with saline-challenged controls (P = 0.0185, ANOVA). C57BL/6 mice were hyporesponsive to methacholine-induced changes in R_L even in the saline-challenged state as compared with either BALB/c or B6D2F1 saline-challenged mice (Figure 1; P = 0.0001, ANOVA). Baseline levels of resistance in the mice recorded before methacholine challenge were not different in the different strains of mice or aerosol groups (mean measured in a liquid scintillation counter, expressed as a percent of total degranulation (Triton X-100 lysis), and corrected for spontaneous release, and the result was referred to as percent degranulation. A response curve was generated for each mouse by plotting percent degranulation against the serum dilution factor (1/dilution).
Wilder, Collie, Wilson, et al.: AHR Dissociated from IgE and Eosinophilia

R\textsubscript{L} ± SEM in cm H\textsubscript{2}O/ml/sec: BALB/c: saline: 0.702 ± 0.092, OVA: 0.579 ± 0.051; B\textsubscript{6}D\textsubscript{2}F1: saline: 0.763 ± 0.055, OVA: 0.85 ± 0.078; C57BL/6: saline: 0.829 ± 0.064, OVA: 0.879 ± 0.116. Also, the response of BALB/c OVA-immune mice receiving saline aerosols was indistinguishable from that of naive mice receiving no aerosol (data not shown).

**Bronchoalveolar Cellularity**

The number and types of cells recovered from a shallow (0.3 ml) TBL was assessed in OVA-sensitized and OVA- or saline-challenged mice 3 d after aerosol challenge, at the time of peak exhibition of AHR (in the case of the BALB/c mice). The shallow lavage was used to avoid heavy contamination by alveolar macrophages. From 50-75% of the instilled volume was routinely recovered and did not differ among strains of mice or aerosol treatment groups. BALB/c mice appeared to recruit fewer numbers of cells to the airways after OVA aerosol exposure than did the two other strains of mice (Figure 2). However, statistical analysis of the data failed to show a significant overall interaction effect between the strain of mouse and aerosol treatment received (\(P = 0.07\) by two-way ANOVA). BALB/c mice also showed no increase in airway eosinophilia after OVA aerosol challenge (Figure 3a). B\textsubscript{6}D\textsubscript{2}F1 mice recruited significantly more eosinophils into the airways after OVA aerosol exposure than either of the other two strains of mice (two-way ANOVA, \(P = 0.0009\) indicates an interaction between strain and aerosol treatment; \(P = 0.0005\) reveals the difference between the strains and \(P = 0.0012\) reveals the difference between the aerosol groups). In B\textsubscript{6}D\textsubscript{2}F1 mice, the eosinophils accounted for an average of 57.3% ± 6.5% (mean ± SEM) of the TBL cells, whereas C57BL/6 mice had an average of 24.3% (± 4.1%) eosinophils and BALB/c mice had 2.3% (± 1.5%) eosinophils after OVA aerosol challenge. All three strains of mice had an average 0.49% (± 0.22%) eosinophils in their TBL after saline aerosol exposure, with a range of means from 0.1% (± 0.072%) for BALB/c mice to 0.79% (± 0.51%) for B\textsubscript{6}D\textsubscript{2}F1 mice. Neither the number of cells nor cell types recovered from bronchoalveolar lavage of naïve mice differed from those of saline aerosol exposed, OVA-immune mice.

![Figure 1](image1.png)  **Figure 1.** BALB/c, but not B\textsubscript{6}D\textsubscript{2}F1 or C57BL/6 mice, show increased pulmonary resistance after OVA–alum immunization and a single day of OVA aerosol challenge as compared with saline-challenged OVA-immune controls (\(P = 0.0185\), ANOVA). All mice were immunized intraperitoneally with OVA in alum and were aerosol-treated with either OVA (filled diamonds) or saline (open diamonds) as described in the MATERIALS AND METHODS. Changes in lung resistance (R\textsubscript{L}) with increasing doses of intravenously delivered methacholine were measured in anesthetized, mechanically ventilated mice as described. Statistical differences between OVA- and saline-challenged groups of mice at individual methacholine doses are noted by asterisks (unpaired \(t\) tests, \(P < 0.05\)). Data represent the mean change from baseline for individual mice: BALB/c: saline: \(n = 14\), OVA: \(n = 12\); B\textsubscript{6}D\textsubscript{2}F1: saline: \(n = 12\), OVA: \(n = 11\); C57BL/6: saline: \(n = 23\), OVA: \(n = 19\).

![Figure 2](image2.png)  **Figure 2.** Influx of cells into the airways of OVA-immune mice that received an aerosol challenge with either saline (open bars) or OVA (solid bars). Data represent the mean cell number (± SEM) recovered in the TBL from 15–34 mice in each aerosol-challenged group.
Pulmonary Inflammation

The extent and anatomic site of pulmonary inflammation induced by aerosol challenge was assessed 3 d after saline or OVA exposure, at the time at which BALB/c mice exhibited peak changes in AHR, using the scoring system described in MATERIALS AND METHODS. Although OVA-challenged mice of each strain mounted a pulmonary inflammatory response that was significantly greater than that of their saline-challenged counterparts (Figure 4; P < 0.001 comparing the effect of aerosol treatment by two-way ANOVA), all three mouse strains studied had significant increases (asterisks) in pulmonary inflammation after receiving OVA, as compared with saline aerosols (P < 0.001). Total lung scores of naive mice did not differ from those of OVA-immune, saline aerosol-challenged controls (data not shown). The data represent the mean (± SEM) total lung inflammatory score for eight to 14 mice in each aerosol-challenged group.

Antibody Responses

Three days after OVA aerosol challenge, OVA-specific IgG1 and total serum IgE levels in each of the three strains of OVA-immune mice were quantitated by ELISA (Figure 6). All three strains of mice had increased levels of OVA-specific IgG1 and total serum IgE after OVA-alum immunization and treatment with OVA aerosol as compared with nonimmune mice. Levels of antibody in mice exposed to OVA aerosols were compared through one-way ANOVA. Although significant differences between the responses of the three strains of mice were revealed (P = 0.0176), BALB/c mice produced amounts of OVA-specific IgG1 similar to those produced by the other two strains of mice. B6D2F1 mice, however, produced more OVA-specific IgG1 after OVA aerosol than did similarly treated C57BL/6 mice, even though B6D2F1 mice did not manifest AHR after OVA aerosol challenge.

When levels of total serum IgE were similarly analyzed, significant differences between the responses of the three strains of mice were also apparent (P = 0.0267, one-way ANOVA). OVA-challenged BALB/c mice had significantly lower levels of total IgE than did OVA-challenged C57BL/6 mice (P = 0.0084), although it was the latter strain that failed to exhibit AHR.

OVA-specific IgE levels were quantified by measuring the ability of sera from individual OVA-immune mice to sensitize IgE-receptor (FcεR1)-bearing RBL-2H3 cells for the release of [3H]-serotonin upon crosslinking with OVA.
In our experience, this bioassay is more sensitive and reproducible than ELISA for measuring changes in OVA-specific IgE. All three strains of mice demonstrated detectable OVA-specific IgE after OVA aerosol challenge (Figure 7). OVA-challenged BALB/c mice had significantly less circulating OVA-specific IgE than did OVA-challenged B6D2F1 mice ($P < 0.0067$), and had levels equivalent to those of OVA-challenged C57BL/6 mice as measured with one-way ANOVA ($P < 0.0001$ for the effect of strain).

Figure 5. Representative pulmonary pathology after saline (a) (a C57BL/6 mouse is represented) or OVA (b–d) aerosol challenge of BALB/c (b), B6D2F1 (c), or C57BL/6 (d) mice. Arrowheads indicate areas of inflammation. These sections were selected to represent the average inflammatory pattern of each strain of mouse after administering either saline or OVA aerosol challenges (i.e., OVA-challenged BALB/c mice had an average lung inflammatory score of 4 [sum of inflammation around bronchioles, veins, and arteries], which equates to a 1.33 score, or slightly more than 10% of the circumference of bronchioles, arteries, and veins involved with any type of inflammation; similarly, OVA-challenged C57BL/6 and B6D2F1 mice had scores of 2.5 and 2.5, which equated to approximately 20% and 37.5% involvement, respectively). The bar in the lower left of each panel represents $1 \mu$m.

Figure 6. Serum IgG$_1$ anti-OVA (a) and total IgE (b) levels after OVA aerosol challenge of OVA-immune mice. (a) All three mouse strains studied produced increased levels of OVA-specific IgG$_1$ as compared with what found in normal mouse serum (NMS) (BALB/c NMS: $31.97 \pm 11.56$ U/ml; C57BL/6 NMS: $27.01 \pm 2.79$ U/ml; B6D2F1 NMS: $33.2$ U/ml). B6D2F1 mice had more OVA-specific IgG$_1$ than C57BL/6 mice when the two strains were compared with one-way ANOVA ($P = 0.0046$). Data represent the average units (determined as described in MATERIALS AND METHODS) of IgG$_1$ anti-OVA ($\pm$ SEM) antibody for 16–57 mice in each aerosol-challenged group. (b) All three mouse strains produced increased levels of serum IgE as compared with that found in normal mouse serum (BALB/c NMS: $38.11 \pm 11.9$ ng/ml; B6D2F1 NMS: $184.46 \pm 64.86$ ng/ml; C57BL/6 NMS: $61.1 \pm 11.1$ ng/ml). BALB/c mice had significantly less total IgE in their serum than did C57BL/6 mice after OVA aerosol challenge. Data represent the mean concentration of IgE, in ng/ml ($\pm$ SEM) of 15–43 mice in each aerosol-challenged group. *Significant differences between groups as compared by one-way ANOVA ($P < 0.05$).
Discussion

We found that a limited number of OVA–alum systemic sensitizations (Days 0 and 5), and two separate, 60-min OVA aerosol challenges (Day 12) induced BALB/c mice, but not C57BL/6 or B6D2F1 mice, to exhibit AHR (Day 15). The ability to develop AHR did not correlate with the extent of airway eosinophilia, quantifiable pulmonary inflammation, or serum levels of total IgE, OVA-specific IgE, or OVA-specific IgG1, since the magnitude of these responses was either equivalent or greater in one or both of the nonresponding strains of mice as compared with the responding BALB/c strain. OVA-immune BALB/c mice responded to OVA challenge with: (1) recruitment of fewer cells (and significantly fewer eosinophils) to the airways than in B6D2F1 mice after OVA challenge; (2) demonstrating significantly less pulmonary inflammation than OVA-challenged B6D2F1 mice, but equivalent inflammation to OVA-challenged C57BL/6 mice; (3) producing levels of OVA-specific IgG1 equivalent to those of both OVA-challenged B6D2F1 mice and C57BL/6 mice; (4) producing significantly less total IgE than OVA-challenged C57BL/6 mice; and (5) producing less OVA-specific IgE than OVA-challenged B6D2F1 mice, but more than OVA-challenged C57BL/6 mice.

To our knowledge, this is the first description of AHR developing after only a single day of OVA aerosol challenge in systemically immunized mice. We have not yet determined whether the small but insignificant increase in pulmonary eosinophilia or the significant increase in pulmonary inflammation and serum levels of total IgE and OVA-specific IgE and IgG1 observed in OVA-challenged BALB/c mice is required for the development of AHR. However, our data do suggest that increased airway eosinophilia, increased pulmonary inflammation, and high levels of OVA-specific IgG1 or IgE are insufficient to lead directly to AHR in B6D2F1 and C57BL/6 mice. In addition, we clearly show that AHR can develop in BALB/c mice in the absence of a significant increase in airway eosinophilia.

Our data confirm and extend observations made by several other investigators. Kung and coworkers (10) initially immunized B6D2F1 mice with the same immunization protocol as we used, and showed that OVA-challenged mice exhibited significant increases in airway eosinophilia and total serum IgE levels; however, these authors did not report on the induction of AHR or levels of OVA-specific immunoglobulin in the animals’ sera. Additionally, our data emphasize the relative ease with which BALB/c mice, as compared with C57BL/6 mice, develop AHR in response to OVA priming and pulmonary challenge, as was noted by both Zhang and colleagues (12) and Corry and associates (6).

Although the particular immunization protocol used in our study failed to induce significant increases in pulmonary eosinophilia in BALB/c mice, these mice can clearly respond to other methods of OVA immunization and challenge by recruiting greater numbers of eosinophils and pulmonary eosinophilia and total serum IgE levels; however, these authors did not report on the induction of AHR or levels of OVA-specific immunoglobulin in the animals’ sera. Additionally, our data emphasize the relative ease with which BALB/c mice, as compared with C57BL/6 mice, develop AHR in response to OVA priming and pulmonary challenge, as was noted by both Zhang and colleagues (12) and Corry and associates (6).

Although the particular immunization protocol used in our study failed to induce significant increases in pulmonary eosinophilia in BALB/c mice, these mice can clearly respond to other methods of OVA immunization and challenge by recruiting greater numbers of eosinophils to the airways than we observed (6, 13). In addition to the two intraperitoneal inoculations that we used, coupled with multiple pulmonary challenges (intranasal delivery of OVA in saline on three successive days), we also included one sensitization with OVA in alum via the nares, in addition to the two intraperitoneal inoculations that we used, coupled with multiple pulmonary challenges (intranasal delivery of OVA in saline on three successive days). When similarly sensitized BALB/c mice were exposed to only one intranasal OVA challenge, Zhang and colleagues (12) did not find a relative lack of airway eosinophilia in BALB/c mice as compared with C57BL/6 mice. However, the immunization protocol that they used included one sensitization with OVA in alum via the nares, in addition to the two intraperitoneal inoculations that we used, coupled with multiple pulmonary challenges (intranasal delivery of OVA in saline on three successive days). When similarly sensitized BALB/c mice were exposed to only one intranasal OVA challenge, Zhang and colleagues found much reduced eosinophil numbers in the airways. Alternately, it is possible that eosinophils were indeed present in the cytospin preparations of BALB/c TBL cells, but that we were unable to detect them with the Diff-Quik staining method because they were degranulated. How-

Figure 7. OVA-specific IgE in serum of OVA-aerosol-challenged OVA-immune mice. OVA-challenged B6D2F1 mice had significantly greater levels of OVA-specific IgE than did either BALB/c (P = 0.0067) or C57BL/6 (P = 0.0001) mice after OVA challenge. The data are expressed as the percent release of [3H]-serotonin from RBL-2H3 cells after incubation with 5%, 2.5%, or 1.25% serum from individual mice followed by the addition of OVA to crosslink the high-affinity IgE receptor (see MATERIALS AND METHODS). Spontaneous release of [3H]-serotonin in the presence of each serum was uniformly low (< 4.1%). Pooled normal mouse serum from the three strains of mice failed to stimulate degranulation in response to OVA when this sera was tested at a dilution factor of 20. Differences between strains of mice and aerosol-challenged groups were assessed with ANOVA statistics.
ever, we believe this to be unlikely. Morphologically, such cells would appear as though they were neutrophils, and we did not observe an increased number of these cells in cytospin preparations of BALB/c TBL cells. Furthermore, in two instances in which other investigators observed AHR (14, 15), lung eosinophils failed to show morphologic evidence of degranulation as evidenced by electron microscopic techniques, suggesting that AHR can occur in the absence of eosinophil degranulation.

The role of eosinophils in mediating AHR has been extensively studied with murine models and remains controversial. Some studies have suggested that eosinophils are neither required nor sufficient to induce AHR (6–9), whereas others postulate that airway eosinophilia is essential to the manifestation of AHR (2–5). Our studies fail to support a role for eosinophils in contributing to antigen-induced AHR, at least in BALB/c mice, and represent the first instance in which airway eosinophilia has been directly compared in three strains of mice to show that statistical differences between airway eosinophilia exist but cannot explain either the development of AHR in OVA-challenged BALB/c mice or the failure of AHR to develop in the eosinophil-rich environment of OVA-challenged C57BL/6 or B6D2F1 lungs.

The role that IgE plays in causing AHR is also under intense study. We show here that high levels of total or OVA-specific IgE in the sera of C57BL/6 and B6D2F1 mice are insufficient to cause AHR. These data are supported by recent studies suggesting that AHR can develop in the absence of OVA-specific IgE in both OVA-immunized interleukin (IL)-4−/− mice (8) and in mice that received transferred OVA-specific T helper 2 clones that mediated AHR in OVA-challenged mice (4).

It was previously shown that naive, unimmunized BALB/c mice, as compared with C57BL/6 mice, exhibit increased AHR in response to nonspecific bronchoconstricting agents (11). The precise genetic elements that determine AHR are unclear (16, 17). Recently, it has been shown that intrinsic, nonatopic AHR in nonimmune mice is critically dependent on T lymphocytes (18), potentially suggesting that immunity to environmental antigens without ongoing inflammation induces AHR in one strain of mouse but not another. T cells have also been shown to play a crucial role in the development of antigen-driven AHR, as evidenced by an abolishment of AHR when CD4+ T cells are depleted (19) or when their activation is inhibited (20). T cells also accumulate in the lungs (21) and draining lymph nodes (22, 23) after pulmonary antigen challenge. Intriguingly, the role of these T cells in causing AHR may be distinct from the secretion of IL-4 or IL-5 (8). Interferon-γ-secreting CD8+ T cells have also been shown to play a role in downregulating antigen-induced AHR (24).

Our findings and conclusions are consistent with the hypothesis that an OVA-specific T cell or T cell factor may sensitize the smooth muscle of BALB/c but not that of C57BL/6 or B6D2F1 mice to respond with contraction to lower concentrations of methacholine. These T cells or their products may also account for the discordance in levels of IgE and eosinophilia in these three mouse strains. Alternatively, a gene or genes may simply program the smooth muscle of BALB/c mice to respond more vigorously to minimal levels of IgE or eosinophil products, although studies demonstrating development of AHR in IL-5 (6–9)− and/or IgE− (4, 8)−deficient mice speak against this concept. Ultimately, AHR development in mice is most likely under the control of many different factors, including, in some models, pulmonary eosinophilia and levels of serum IgE, whereas in others it is under the control of genetically determined and environmental influences. An intriguing example of this was recently shown by E um and coworkers (14), who concluded that eosinophils were necessary but not sufficient to induce AHR, and acted only in concert with high titers of IgE to induce AHR in a strain of mouse (BP2) specifically bred to produce high levels of IgE.

Our data suggest that even in antigen-sensitized and -challenged mice, the genetic background of the mouse may better predict the ability to develop AHR than do either pulmonary eosinophilia or IgE. Zhang and colleagues (12) reached similar conclusions after finding that OVA-alum immunized BALB/c mice had increased bronchoconstrictive responses to intravenously delivered methacholine than did C57BL/6 mice. In their study, however, the BALB/c mice also responded to the immunization protocol with increased levels of airway eosinophilia, leaving open the possibility that airway eosinophilia may play a role in the induction of AHR. We show here, however, that BALB/c mice can manifest AHR after OVA challenge in the absence of any significant increase in airway eosinophilia. Further, in having compared the development of AHR in BALB/c mice with that in B6D2F1 mice, we can conclusively state that high levels of airway eosinophilia, pulmonary inflammation, total IgE, OVA-specific IgE, or OVA-specific IgG, cannot directly lead to AHR in the B6D2F1 strain of mouse.

Acknowledgments: The authors wish to acknowledge the excellent technical help of Barbara Forrist, Claudia Pertab, James White, Kenneth Olejar, Jr., Gayneth Olson, Linda Izzo, and Maria Martinez. The authors would also like to thank Dr. Angelo Izzo for his insightful discussion of the results, and are grateful for the statistical help provided by Chris Stidley, James White, and Mark Eichinger. This work was supported by the Specialized Centers of Research grant 5P30 H5156384 from the National Heart, Lung and Blood Institute, the University of New Mexico Research A llocation Committee Cigarette Tax Interest Funds appropriated by the New Mexico State Legislature, the U.S. Department of Energy, Office of Health and Environmental Research under Contract No. DE-AC04-76-07733 from the National Institutes of Health.

References


