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Allergen-induced airway disease is mouse strain dependent

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Whitehead, Gregory S., Julia K. L. Walker, Katherine G. Berman, W. Michael Foster, and David A. Schwartz. Allergen-induced airway disease is mouse strain dependent. *Am J Physiol Lung Cell Mol Physiol* 285: L32–L42, 2003. First published March 7, 2003; 10.1152/ajplung.00390.2002.—We investigated the development of airway hyperreactivity (AHR) and inflammation in the lungs of nine genetically diverse inbred strains of mice [129/SvIm, A/J, BALB/cJ, BTBR+(T)/tf/tf, CAST/Ei, C3H/HeJ, C57BL/6J, DBA/2J, and FVB/NJ] after sensitization and challenge with ovalbumin (OVA). At 24, 48, and 72 h post-OVA exposure, the severity of AHR and eosinophilic inflammation of the mouse strains ranged from relatively unresponsive to responsive. The severity of the airway eosinophilia of some strains did not clearly correlate with the development of AHR. The temporal presence of T helper type 2 cytokines in lung lavage fluid also varied markedly among the strains. The levels of IL-4 and IL-13 were generally increased in the strains with the highest airway eosinophilia at 24 and 72 h postexposure, respectively; the levels of IL-5 were significantly increased in most of the strains with airway inflammation over the 72-h time period. The differences of physiological and biological responses among the inbred mouse strains after OVA sensitization and challenge support the hypothesis that genetic factors contribute, in part, to the development of allergen-induced airway disease.

asthma; airway hyperreactivity; eosinophils; cytokines

ASTHMA IS A CHRONIC inflammatory disease of the airways characterized by reversible airway obstruction, airway hyperreactivity (AHR), and remodeling of the airways. Infiltration of eosinophils in the lungs is a fundamental trait of the inflammatory response in allergic asthma and may be important in the pathogenesis of this disease (6, 12, 38). T lymphocytes are also believed to play a pivotal role in the development of allergic asthma. $CD4^+$ T cells infiltrate the lung lumen and express a T helper type 2 (Th2) pattern of cytokines. Cytokines secreted by Th2 cells, including interleukin (IL)-4, IL-5, and IL-13, appear to function in concert with chemokines and other mediators to recruit and activate the eosinophils of the allergic inflammatory response (22, 43, 45). Activation of the eosinophils leads to their degranulation and release of inflammatory mediators, which may cause extensive tissue damage, propagate airway obstruction, and contribute to the chronic inflammation of the airways that characterizes persistent asthma.

Neutrophils are another participant in allergen-induced asthma (2, 3, 27, 42). Neutrophils are increased in severe asthmatic patients (42). In addition, asthma severity, characterized by forced expiratory volume in 1 s, correlates with the degree of neutrophilia in sputum or bronchial biopsy specimens (19, 27, 31). Neutrophilia in asthma, however, appears to be independent of the Th2 response and is not affected by treatment with anti-IgE (33). The roles of neutrophils in asthma remain unclear, but it has been suggested that increased neutrophils in the airways may contribute to the pathogenesis of chronic airway narrowing and obstruction (46). Neutrophils secrete a variety of inflammatory mediators, including cytokines, proteases, and reactive oxygen species, which may lead to asthmatic symptoms (46).

The etiology of asthma is complex and multifactorial. Many environmental allergens, viral infections, and environmental irritants are known contributors to the development of asthma (7, 20, 22). In addition, genetic factors are also important in the development and severity of this disease (13, 18, 30, 32, 37). When both parents are asthmatic, there is a sevenfold increased chance that the child will develop asthma; when there is only one asthmatic parent, there is only a threefold increased chance that a child will become asthmatic (8). Despite evidence that genetic factors are involved in this disease, the mechanisms that regulate the development and severity still remain unclear.

To better understand the mechanisms underlying the asthmatic response, recent studies have characterized the physiological and inflammatory responses in genetically diverse inbred mouse strains following allergen sensitizations and exposure (1, 26, 35, 40, 44, 48). The inbred strains of mice have been shown to differ in their ability to mount an allergen-induced asthmatic response (1, 26, 35, 40, 44, 48). For example, some mouse strains (e.g., BALB/c, DBA/2, and FVB) have greater AHR, eosinophilia, and IgE production

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after sensitization and challenge, whereas other strains (e.g., A/J, 129/Sv, and C3H/He) fail to produce an allergic response (1). These studies have helped to clarify some of the mechanisms in this disease and further suggest that genetic factors play a role. However, these studies have not assessed many of the mediators of the inflammatory response in the different inbred strains of mice. In addition, these studies have only assessed the specific facets of this complex disease at single points in time following treatment. Since asthma is a very dynamic process, we reasoned that levels of cytokines associated with inhaled ovalbumin (OVA; i.e., IL-4, IL-5, and IL-13) might differ among genetically diverse strains of mice.

In the present investigation, we subjected mice from nine genetically diverse inbred strains [129/SvIm, A/J, BALB/cJ, BTBR+(T)/tf/tf, C3H/HeJ, C57BL/6J, CAST/Ei, DBA/2J, FVB/NJ] to a classic evaluation of allergen-induced asthma. Specifically, we characterized how short-term exposure to OVA correlates with the temporal responses of AHR and inflammation of these mice. A priori, we hypothesized that these genetically diverse strains of mice would manifest very different airway responses to sensitization and challenge with OVA.

MATERIALS AND METHODS

Animals. Thirty-six male mice (6–8 wk) from nine strains [129/SvIm, A/J, BALB/cJ, BTBR+(T)/tf/tf, CAST/Ei, C3H/HeJ, C57BL/6J, DBA/2J, and FVB/NJ] were obtained commercially (Jackson Laboratories, Bar Harbor, ME). The animals were housed in plastic shoebox-type cages suspended over absorbent bedding and were maintained on a 12-h diurnal cycle. Food and water were provided ad libitum.

Sensitization and airway challenge. The mice from each strain were equally divided into two groups: OVA group and control (Alum-only) group. The OVA mice (n = 18) were sensitized on *days 0* and *14* with an intraperitoneal injection of 10.0 μg of OVA (chicken egg, grade V; Sigma, St. Louis, MO) emulsified in 2.0 mg of aluminum hydroxide (Alum; AlumInject; Pierce Chemical, Rockford, IL) in a total volume of 100 μl. Each OVA injection contained ~0.022 μg of lipopolysaccharide (LPS). The Alum mice (n = 18) were sensitized with an intraperitoneal injection of 2.0 mg of Alum in a total volume of 100 μl on the same days.

On *day 20*, airway reactivity to methacholine (MCh) was measured in all mice of each strain, and whole lung lavage fluid was collected from six mice (3 OVA, 3 Alum-only) of each strain. On *day 21*, the remaining 30 mice (15 OVA, 15 Alum-only) of each strain were exposed to aerosolized OVA (1.0% OVA in saline) for 1 h. The mice were placed in a 69-liter whole body exposure chamber with a six-jet nebulizer (9306; Fairchild Industrial Products, Winston-Salem, NC) generating the aerosol. OVA (1.5 g; chicken egg, grade V, Sigma) emulsified in 150 ml of saline was added to the reservoir of the nebulizer. Approximately 24 l/min of filtered air was focused through three of the six jets, which generated the OVA aerosol and diluted with an additional 16 l/min of air before being added to the chamber. The generated aerosol was transferred to the chamber by operating the exposure system in a continuous flow (dynamic) mode. In addition to the aerosolized OVA, the exposure atmosphere contained 0.4 $\mu g/m^3$ of LPS. At 24, 48, and 72 h postexposure, the airway responses to inhaled MCh and airway inflammation were assessed in 10 mice (5 OVA, 5 Alum-only) of each strain.

Determination of airway responsiveness. Estimates of the physiological response to a standard MCh challenge (0 and 10 mg/ml MCh) were collected using whole body plethysmographic techniques, similar to those described previously (14). Briefly, the mice were individually placed in a whole body plethysmograph (PLY3211 V2.1; Buxco Electronics, Sharon, CT) that contained built-in pneumotachographs. The mice were ventilated by 0.8 l/min regulated bias airflow (PLY1040; Buxco Electronics) through the plethysmograph. A differential pressure transducer was used to measure the pressure differential across the pneumotachograph to determine flow. The pressure differential between the inside of the box and a reference space was used. The flow through the pneumotachograph was linear to the differential pressure and was integrated. Transducer signals were conditioned using an amplifier (Max 2270 preamplifier; Buxco Electronics), digitized, and processed in real time. Real-time calculations of frequency and breath waveform [expiratory time (T_e), relaxation time (T_r), peak expiratory flow (PEF), peak inspiratory flow (PIF)] were performed and recorded electronically by computer software (BioSystem XA software; Buxco Electronics). Estimates of airway responsiveness, expressed as enhanced pause (Penh), were derived from the ventilation and flow-derived parameters. Penh = $[(T_e - T_r/T_r) \times (PEF/PIF)]$. Penh values were averaged every 30 s and recorded for a minimum of 3-min intervals at baseline and after stimulation with each concentration of MCh. The different concentrations of aerosolized MCh were administered for 1 min using an ultrasonic nebulizer and nebulizer control unit (Buxco Electronics). The dilution flow through the mixing chamber was 10 l/min.

Whole lung lavage. Whole lung lavage was performed in the OVA and Alum mice immediately after the plethysmographic assessment. Briefly, each animal was euthanized by CO_2 inhalation, and the chest was opened. The trachea was exposed and intubated via PE-90 tubing (0.86-mm inner diameter, 1.27-mm outer diameter). Sterile pyrogen-free saline was infused into the lungs to total lung capacity, which was defined at a pressure of 25 cmH_2O, and was then collected. A total of 6.0 ml of saline was infused into the lungs, and the total volume of retrieved fluid was noted. The cells were then isolated from the whole lung lavage fluid by centrifugation at 2,000 rpm for 10 min. The supernatant was stored at −80°C for later assessment of cytokine levels. The cells were resuspended in 1.0 ml of sterile Hanks' balanced salt solution and counted using a hemacytometer. A 100-μl sample of the suspension from each animal was also centrifuged (Cytospin 3; Shandon, Pittsburgh, PA) to extract cells onto cytoslide (Shandon) preparations. The cells were then stained with hematoxylin and eosin (Hema 3, Wright-Giemsa stain; Biochemical Sciences, Swedesboro, NJ). Differential cell counts of pulmonary inflammatory cells (alveolar macrophages, neutrophils, lymphocytes, and eosinophils) were determined using standard morphological criteria and then the number of eosinophils and neutrophils per milliliter of lavage fluid was calculated.

Cytokine analysis. Concentrations of IL-4 and IL-5 in whole lung lavage fluid were determined using commercial multiplexed fluorescent bead-based immunoassays (Upstate Biotechnology, Lake Placid, NY), similar to those described previously (23). Briefly, standard concentrations of the respective cytokines (50 μl) were placed in duplicate into the wells of a 96-well filtration plate. Standards were one-half dilutions, ranging from 3.9 to 250 pg/ml, of recombinant mouse IL-4 and IL-5. Unknown samples consisting of 50 μl of

whole lung lavage fluid were then added to the additional wells. The samples were incubated with 25 μl of anti-mouse multicytokine beads, specific for mouse IL-4 and IL-5, at 4°C for 18 h on a plate shaker. Unbound cytokine beads were filtered through the wells using a vacuum manifold. Biotin anti-mouse multicytokine reporter was added to each well as a secondary/detection antibody and incubated at 37°C in the dark on a plate shaker for 2 h. Streptavidin-phycoerythrin (25 μl) was added to the wells and incubated at 37°C in the dark on a plate shaker for 2 h. The addition of 25 μl of stop solution terminated the reactions. The samples were read using a Luminex^{100} Instrument (Luminex, Austin, TX) in which a minimum of 50 beads per cytokine for each sample were analyzed. Blank values were subtracted from all readings. The lower limit of sensitivity for IL-4 and IL-5 was 3.0 and 2.0 pg/ml, respectively.

Concentrations of IL-13 in whole lung lavage fluid were determined using commercial ELISA kits specific for mouse IL-13 (R&D Systems, Minneapolis, MN). The lower limit of sensitivity for detection of IL-13 was 1.5 pg/ml.

Statistical analysis. All results were presented as means ± SE. Mann-Whitney's nonparametric *t*-tests were used to compare the treatment groups with their baseline values. ANOVA was used to determine the levels of difference among all groups. Statistical significance was set at $P < 0.05$.

RESULTS

AHR. Basal Penh, as assessed by the response to aerosolized saline (vehicle for MCh), was measured after sensitization, but prior to challenge with OVA aerosol, in both the OVA/Alum- and Alum-only-sensitized mice from each of the nine strains (Fig. 1). As expected, there was little difference in basal Penh in any of the mice regardless of strain or sensitization. In contrast, baseline airway responsiveness to MCh (10 mg/ml) in the OVA-sensitized mice before exposure to aerosolized OVA revealed interesting strain-dependent variations. The A/J and BTBR+(T)/tf/tf strains had the highest Penh values after the MCh challenge, whereas the FVB/NJ and DBA/2J strains had the lowest responses to the MCh challenge. No significant differences in Penh were observed between the OVA/Alum- and Alum-only-sensitized mice in any of the nine strains after the MCh challenge; therefore, sensitization alone had no effect on AHR.

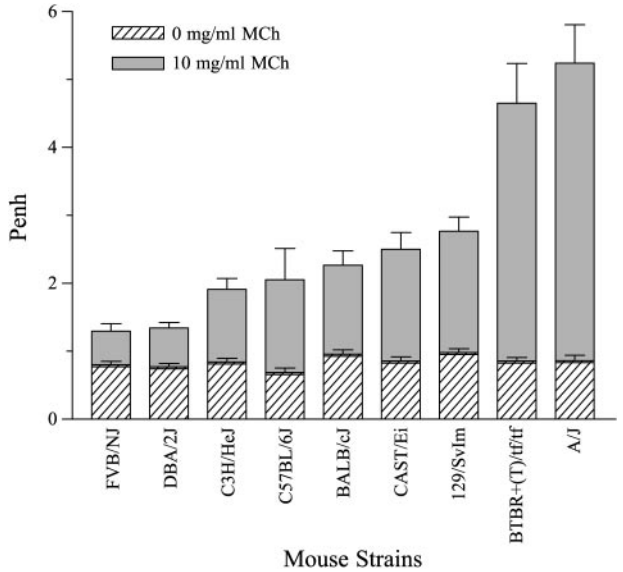

Fig. 1. Baseline values of airway responsiveness in 9 mouse strains sensitized to ovalbumin (OVA). Enhanced pause (Penh) was measured after saline [no methacholine (MCh)] and MCh (10 mg/ml) administration. Values are means ± SE. There were no significant differences between the OVA/aluminum hydroxide (Alum)- and Alum-only-sensitized mice for each strain after administration of saline and MCh. $P < 0.05$.

Airway responsiveness to MCh (10 mg/ml) was measured in OVA-sensitized mice of each strain at 24, 48, and 72 h postexposure and then compared with the respective strain's mean preexposure levels (mean values in Fig. 1) after a 10-mg/ml MCh challenge (Fig. 2). As expected, the mouse strains had variable airway reactivity to the MCh after the aerosolized OVA challenge. The FVB/2J strain demonstrated the greatest increase of AHR following exposure to OVA. The increased responsiveness persisted throughout the postexposure evaluation. The OVA-sensitized mice of the C3H/HeJ, BALB/cJ, DBA/2J, and 129/SvIm strains also demonstrated significantly increased AHR after the OVA challenge. However, the increased airway responsiveness of these strains was time dependent for each strain. The postexposure Penh values of the BTBR+(T)/tf/tf and C57BL/6J strains were increased at least one time point postexposure compared with their preexposure values, but statistical significance was not observed. The A/J and CAST/Ei strains demonstrated decreased Penh values after the OVA challenge; however, this same trend was observed in the Alum-treated mice of these strains. Thus this reduction in Penh is not a specific effect of OVA sensitization and challenge.

Cellular response in whole lung lavage fluid. Whole lung lavage fluid was collected, and total leukocytes were counted in both the OVA- and Alum-only-sensitized mice from each of the nine strains. As shown in Table 1, there were temporal differences in total leukocyte accumulation in the lavage fluid among the strains. For example, the OVA-sensitized mice of the 129/SvIm and CAST/Ei strains had significant increases of leukocytes throughout the postexposure evaluation. The OVA mice of the remaining strains, A/J, BALB/cJ, DBA/2J, C57BL/6J, C3H/HeJ, BTBR+(T)/tf/tf, and FVB/NJ, had significant infiltrations of leukocytes only at select times following OVA challenge. All the OVA-sensitized mice of the nine strains had significantly increased leukocytes at 72 h postexposure.

The OVA-sensitized mice of the different strains also had different degrees of cellular infiltration in the airways (Table 1). The 129/SvIm and C57BL/6J strains had the greatest number of leukocyte infiltrates in lavage fluid among the different strains, ~420,000 and 310,000 cells/ml, respectively, at 72 h postchallenge. Whereas the BTBR+(T)/tf/tf and FVB/NJ strains had moderate increases of leukocytes in the airways throughout the postexposure evaluation, the remaining strains, A/J, BALB/cJ, C3H/HeJ, DBA/2J, and

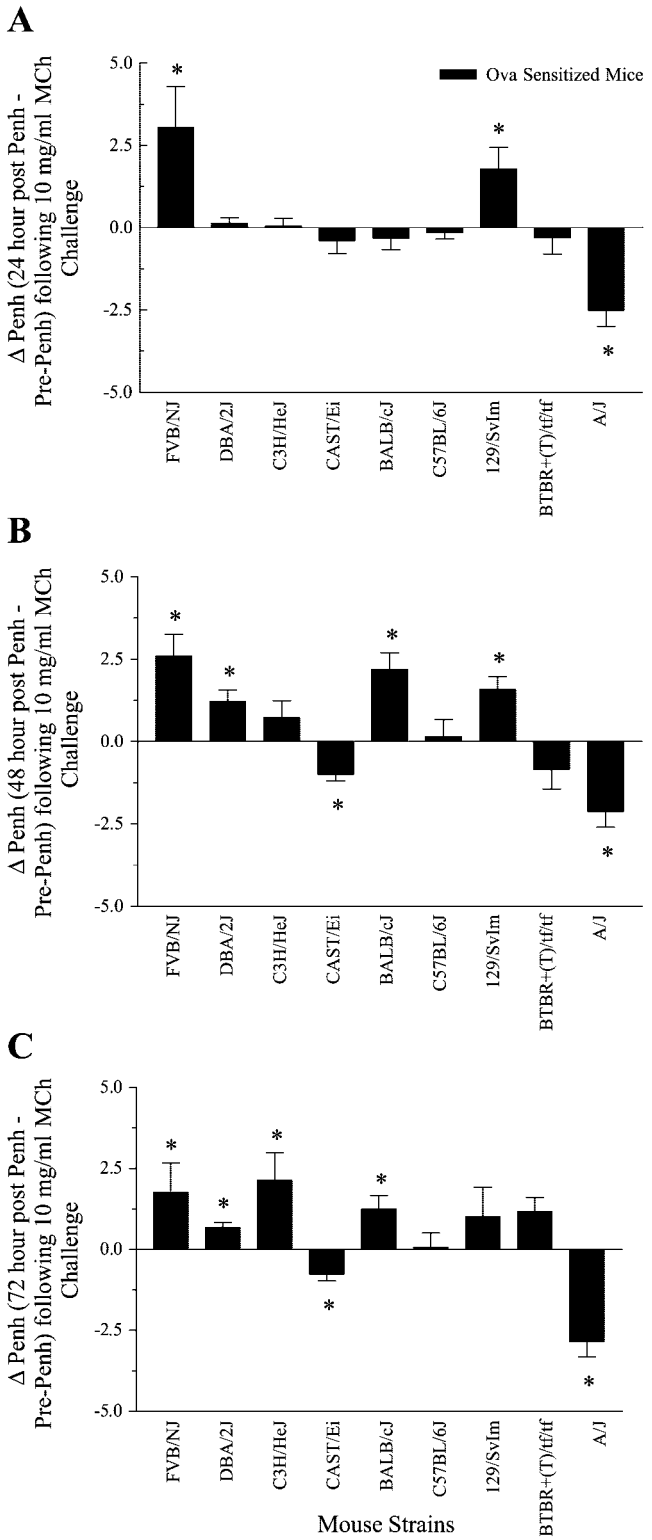

Fig. 2. Changes of airway responsiveness in 9 strains of OVA-sensitized mice following exposure to aerosolized OVA. Values of Penh were measured after administration of 10 mg/ml of MCh at 24 (*A*), 48 (*B*), and 72 (*C*) h post-OVA exposure, and then the respective strain's mean values of preexposure Penh following MCh challenge were subtracted. Values are means ± SE. *Significantly different ($P \leq 0.05$) from preexposure group.

CAST/Ei, had low to moderately low infiltrates into the lung air space throughout the postexposure evaluation.

Eosinophils contributed to the elevations in the total leukocytes recruited to the airways. Before the aerosol OVA challenge, the mice from each strain exhibited <1% eosinophils. As seen in Table 1, the eosinophil accumulation in the lavage fluid of the OVA-sensitized, OVA-challenged mice significantly increased, and for most strains, the number of eosinophils was significantly increased throughout the postexposure evaluation. Only the C3H/HeJ, BALB/cJ, and C57BL/6J strains did not have significant differences of eosinophils between the OVA- and the Alum-only-sensitized mice throughout the evaluation. However, there were variations in the number of eosinophils that infiltrated to the lung lumens among the OVA-sensitized mice of each strain (Table 1). After the aerosolized OVA challenge, the 129/SvIm and C57BL/6J strains had the largest influx of eosinophils to the airways, which was most prevalent at 72 h postexposure. In contrast, the A/J, BALB/cJ, CAST/Ei, and C3H/HeJ strains showed only a modest eosinophil response of <2,000 eosinophils/ml throughout the postexposure evaluation.

Wide ranges of neutrophils were infiltrated in the air space of the strains after OVA sensitization and exposure to aerosolized OVA (Table 1). For example, five strains, CAST/Ei, A/J, DBA/2J, C57BL/6J, and 129/SvIm, had significantly increased neutrophils in the lavage fluid. The highest number of neutrophil infiltrations in the lavage fluid was found in the C57BL/6J, CAST/Ei, and 129/SvIm strains. These strains had neutrophils in the air space that were highest at 48 h postexposure. The A/J and DBA/2J also had significantly increased neutrophilia at 24 h postexposure. However, the number of neutrophils in the lung airways was of lesser magnitude compared with higher neutrophil responders. The remaining strains, BALB/cJ, C3H/HeJ, and FVB/NJ, showed a weak neutrophil response throughout the postexposure evaluation.

Expression of cytokines in the airways. As shown in Table 2, cytokine expression measured after the OVA challenge varied markedly among the strains. For instance, only six of the nine strains sensitized to OVA, 129/SvIm, A/J, BTBR+(T)/tf/tf, BALB/cJ, C57BL/6J, and DBA/2J, had significant increases of IL-4 expression in the lavage fluid at one or more time points during the postexposure evaluation. In addition, among these six strains, only BTBR+(T)/tf/tf mice had significantly increased expression of IL-4 throughout the postexposure evaluation. The 129/SvIm, A/J, and C57BL/6J strains had increased expression at two time points after the OVA exposure, and the DBA/2J and BALB/cJ strains had increased expression at only one time point. Each of these six strains had significantly increased expression of IL-4 at 24 h postexposure.

The levels of IL-5 were significantly increased in the OVA-sensitized, OVA-challenged mice of seven of the nine strains compared with the Alum-only-sensitized, OVA-challenged mice (Table 2). Four of these strains, 129/SvIm, A/J, C57BL/6J, and BTBR+(T)/tf/tf, had in-

Table 1. *Total number of leukocytes and eosinophil and neutrophil profile of whole lung lavage fluid after OVA sensitization and aerosol challenge*

	Total Leukocytes/ml, $\times 10^4$			Eosinophils/ml, $\times 10^4$			Neutrophils/ml, $\times 10^4$		
	Hours postexposure			Hours postexposure			Hours postexposure		
Mouse Strain	24	48	72	24	48	72	24	48	72
C3H/HeJ	1.1 ± 0.4	1.2 ± 0.2*	1.4 ± 0.3*	0.2 ± 0.1	0.3 ± 0.1*	0.6 ± 0.2*	≤ 0.1	≤ 0.1	≤ 0.1
BTBR+(T)/tf/tf	10.3 ± 2.9	11.1 ± 4.9*	4.9 ± 0.8*	1.9 ± 1.1*	4.4 ± 2.6*	0.6 ± 0.3*	4.4 ± 1.5	1.1 ± 7.0	0.9 ± 0.3
CAST/Ei	5.2 ± 4.0*	5.2 ± 3.9*	2.0 ± 0.7*	0.4 ± 0.2*	0.3 ± 0.1*	1.0 ± 0.4*	2.8 ± 2.5*	3.5 ± 3.2	0.1 ± 0.1
A/J	3.6 ± 0.7*	1.9 ± 0.4	4.0 ± 0.8*	1.3 ± 0.4*	0.7 ± 0.2*	1.6 ± 0.3*	0.2 ± 0.1*	≤ 0.1	≤ 0.1
DBA/2J	3.7 ± 0.7*	6.6 ± 3.1	6.4 ± 1.5*	1.1 ± 0.5*	5.2 ± 2.7*	2.1 ± 0.8*	0.4 ± 0.1*	≤ 0.1	≤ 0.1
FVB/NJ	2.5 ± 0.9	10.3 ± 1.6*	7.7 ± 3.9*	0.3 ± 0.2*	5.5 ± 0.6*	3.3 ± 1.3*	≤ 0.1	0.3 ± 0.2	≤ 0.1
BALB/cJ	1.5 ± 0.3	2.4 ± 0.6	5.7 ± 1.5*	0.4 ± 0.2	0.40 ± 0.13*	3.27 ± 1.23*	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
C57BL/6J	2.4 ± 0.6*	15.7 ± 7.4	31.1 ± 12.0*	0.4 ± 0.2*	6.5 ± 3.6	25.5 ± 9.6*	0.3 ± 0.1*	4.8 ± 2.3*	1.6 ± 1.1
129/SvIm	8.1 ± 3.9*	21.4 ± 4.6*	42.2 ± 18.7*	5.7 ± 3.3*	17.9 ± 4.8*	27.6 ± 11.6*	0.7 ± 0.3*	0.9 ± 0.3*	2.0 ± 0.9

Values are means ± SE. Mice of various strains were sensitized with ovalbumin (OVA)/aluminum hydroxide (Alum) and then challenged with aerosolized OVA. Whole lung lavage fluid was obtained for analysis of cell counts and differentials in the lung's air space at 24, 48, and 72 h following OVA exposure. Mouse strains were ranked according to the eosinophil response in the OVA-sensitized mice at 72 h postexposure. *Significantly different ($P \leq 0.05$) from Alum group.

creased expression throughout the postexposure evaluation. The FVB/NJ and DBA/2J strains had significantly increased expression of IL-5 in the airways at two time points postexposure, whereas the C3H/HeJ strain only had significantly increased levels of IL-5 at one time point after the OVA challenge. Each of these seven strains had significantly increased expression of IL-5 at 24 h postexposure.

Mice of the different strains also varied in their expression of IL-13 in the lung airways measured after OVA sensitization and aerosol challenge (Table 2). Mice of four strains, 129/SvIm, BALB/cJ, C57BL/6J, and FVB/NJ, exhibited significantly increased levels of IL-13. IL-13 was significantly elevated at 24 h postexposure for the FVB/NJ strain and at 72 h postexposure for the BALB/cJ and C57BL/6 strains. IL-13 was significantly elevated at 48 and 72 h postexposure for the 129/SvIm strain.

Comparison of airway responsiveness and concentration of eosinophils in the airways. To explore the relationships between allergen-induced AHR and airway eosinophilia among the nine strains, and to identify strains with different lung responses to OVA, cosegregation plots were generated for each time point throughout the postexposure evaluation (Fig. 3). The eosinophils collected in the whole lung lavage fluid of each strain were plotted against each strain's Penh values after the MCh challenge. The severity of the eosinophilic inflammation developed in the airways of some strains did not necessarily correlate with the physiological response of the strain to inhaled OVA. For instance, the 129/SvIm and C57BL/6J strains exhibited the highest increases of eosinophil infiltrates to the airways; however, only the 129/SvIm strain developed increased airway reactivity postexposure. The FVB/NJ strain, which had only moderate eosinophilic inflammation that was not as severe as the C57BL/6J strain, developed increased airway responsiveness at all times postexposure. In addition, the BALB/cJ, BTBR+(T)/tf/tf, DBA/2J, and C3H/HeJ strains each had a weak-to-moderate eosinophil response to the OVA sensitization and challenge, but only three of

Table 2. *Expression of Th2 cytokines (IL-4, IL-5, and IL-13) in whole lung lavage fluid after OVA sensitization and aerosol challenge*

	IL-4 pg/ml			IL-5 pg/ml			IL-13 pg/ml		
	Hours postexposure			Hours postexposure			Hours postexposure		
Mouse Strain	24	48	72	24	48	72	24	48	72
C3H/HeJ	n.d.	n.d.	n.d.	13.6 ± 1.8*	4.6 ± 1.5	11.6 ± 2.1	n.d.	n.d.	n.d.
BTBR+(T)/tf/tf	28.8 ± 18.5*	28.3 ± 15.7*	17.3 ± 3.7*	43.1 ± 10.4*	29.6 ± 3.2*	37.6 ± 12.2*	n.d.	n.d.	n.d.
CAST/Ei	n.d.	3.0 ± 1.8	n.d.	21.1 ± 4.8	14.5 ± 1.7	11.0 ± 3.3	n.d.	n.d.	n.d.
A/J	24.9 ± 3.8*	4.2 ± 0.3*	n.d.	38.3 ± 6.5*	22.1 ± 2.1*	8.4 ± 2.4*	n.d.	n.d.	n.d.
DBA/2J	45.8 ± 19.7*	n.d.	4.2 ± 1.4	11.4 ± 0.6*	10.0 ± 2.0*	4.5 ± 1.9	n.d.	n.d.	n.d.
FVB/NJ	6.8 ± 3.0	7.3 ± 2.5	5.7 ± 1.8	43.1 ± 8.5*	44.6 ± 4.8*	16.8 ± 3.0	n.d.	11.5 ± 3.5*	8.5 ± 8.0
BALB/cJ	11.0 ± 3.4*	3.3 ± 1.7	n.d.	38.3 ± 20.7	11.6 ± 4.1	37.7 ± 27.5	11.6 ± 8.5	3.7 ± 2.8	14.0 ± 5.3*
C57BL/6J	6.0 ± 1.8*	7.5 ± 3.0*	4.1 ± 0.5	21.3 ± 1.8*	18.4 ± 2.0*	51.8 ± 35.6*	n.d.	10.2 ± 7.0	67.1 ± 35.6*
129/SvIm	35.3 ± 12.2*	6.9 ± 2.8	6.3 ± 1.0*	34.4 ± 20.4*	51.3 ± 14.6*	55.9 ± 25.0*	6.6 ± 6.1	16.1 ± 5.6*	48.0 ± 20.6*

Values are means ± SE. Mice of various strains were sensitized with OVA/Alum and then challenged with aerosolized OVA. Whole lung lavage fluid was obtained for analysis of cytokine expression (IL-4, IL-5, and IL-13) in the lung's airways at 24, 48, and 72 h postexposure. n.d., not detectable. Mouse strains were ranked according to the eosinophil response in the OVA-sensitized mice at 72 h postexposure (Table 1). *Significantly different ($P \leq 0.05$) from Alum group.

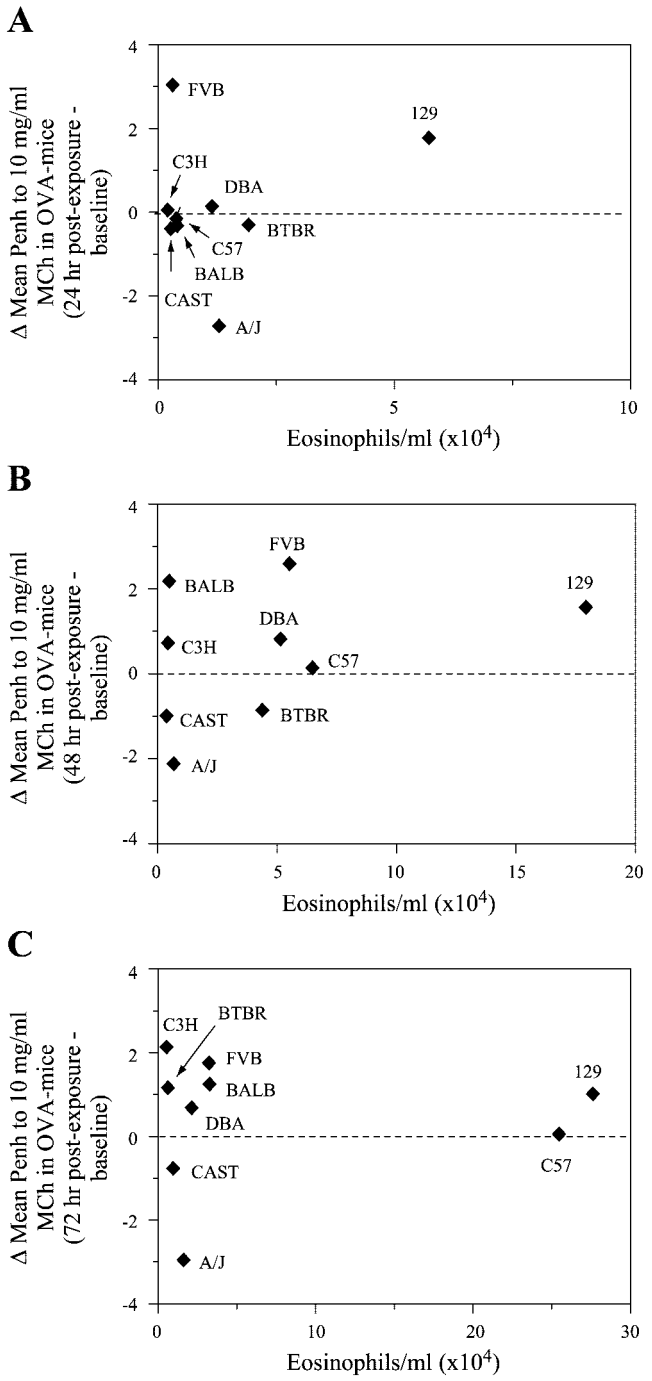

Fig. 3. Cosegregation plots for the mean Penh differences to MCh (10 mg/ml) challenge between the post- and preexposure OVA mice vs. the mean number of eosinophils per milliliter recovered from whole lung lavage fluid of OVA mice. The cosegregation plots were developed for data collected at 24 (*A*), 48 (*B*), and 72 (*C*) h postexposure. Note that the eosinophil scales (*x*-axis) are different for each time point. Data are presented as mean values for each strain.

these four strains (BALB/cJ, DBA/2J, and C3H/HeJ) had enhanced AHR. Furthermore, the A/J and CAST/Ei strains had similar eosinophil infiltrates to the airways as the BALB/cJ, DBA/2J, and C3H/HeJ strains, but these two strains had decreased airway responsiveness after OVA stimulation.

Comparison of eosinophil infiltrates and Th2 cytokines (IL-4, IL-5, and IL-13). To explore the relationships between airway eosinophilia and the expression of Th2 cytokines (IL-4, IL-5, and IL-13) among the nine strains, cosegregation plots were generated for each time point throughout the postexposure evaluation. IL-4 was an early response cytokine that was increased in the mouse strains with the highest eosinophil infiltrates at 24 h postexposure (Fig. 4). However, the FVB/NJ strain, which also had significant increased eosinophilia at 24 h post-OVA challenge, did not have increased expression of IL-4. At 48 and 72 h postexposure, the expression of IL-4 was near control levels in the airways of most of the strains. At these time points, the expression of IL-4 did not clearly correlate with the airway eosinophilia.

The expression of IL-5 coincided with the infiltration of eosinophils for many, but not all, of the strains (Fig. 5). Elevated levels of IL-5 were present in the lung lavage for each strain at each time point postexposure in which the eosinophils per milliliter in the lavage fluid exceeded 4.0×10^4. In addition, the C57BL/6J and 129/SvIm strains had the highest numbers of eosinophils and levels of IL-5 in the airways at 72 h postexposure. However, the expression of IL-5 did not clearly correlate with the airway eosinophilia of some of the strains throughout the postexposure evaluation. For instance, the BALB/cJ and CAST/Ei strains, which had increased eosinophilia, did not have increased expression of IL-5 at any time throughout the postexposure evaluation.

IL-13 was generally increased during the later stages of the postexposure evaluation (Fig. 6). Its increased expression correlated with the mouse strains that had the highest airway eosinophilia during this time period.

Comparison of airway responsiveness and concentration of neutrophils in the airways. To explore the relationships between allergen-induced AHR and airway neutrophilia among the nine strains of mice, cosegregation plots were generated for each time point throughout the postexposure evaluation (Fig. 7). The neutrophils collected in the whole lung lavage fluid of each strain were plotted against each strain's Penh values following the MCh challenge. The severity of the neutrophilic inflammation developed in the airways of some strains did not necessarily correlate with the development of AHR. For instance, the C57BL/6J and CAST/Ei strains, which had the highest airway neutrophilia among the strains after the OVA aerosol challenge, did not develop AHR. In addition, of the five strains that developed AHR (FVB/NJ, BALB/cJ, DBA/2J, C3H/HeJ, and 129/SvIm), only two strains (DBA/2J and 129/SvIm) had significant infiltration of neutrophils in the airways.

DISCUSSION

Our results demonstrate that genetically diverse strains of mice respond differently in the development

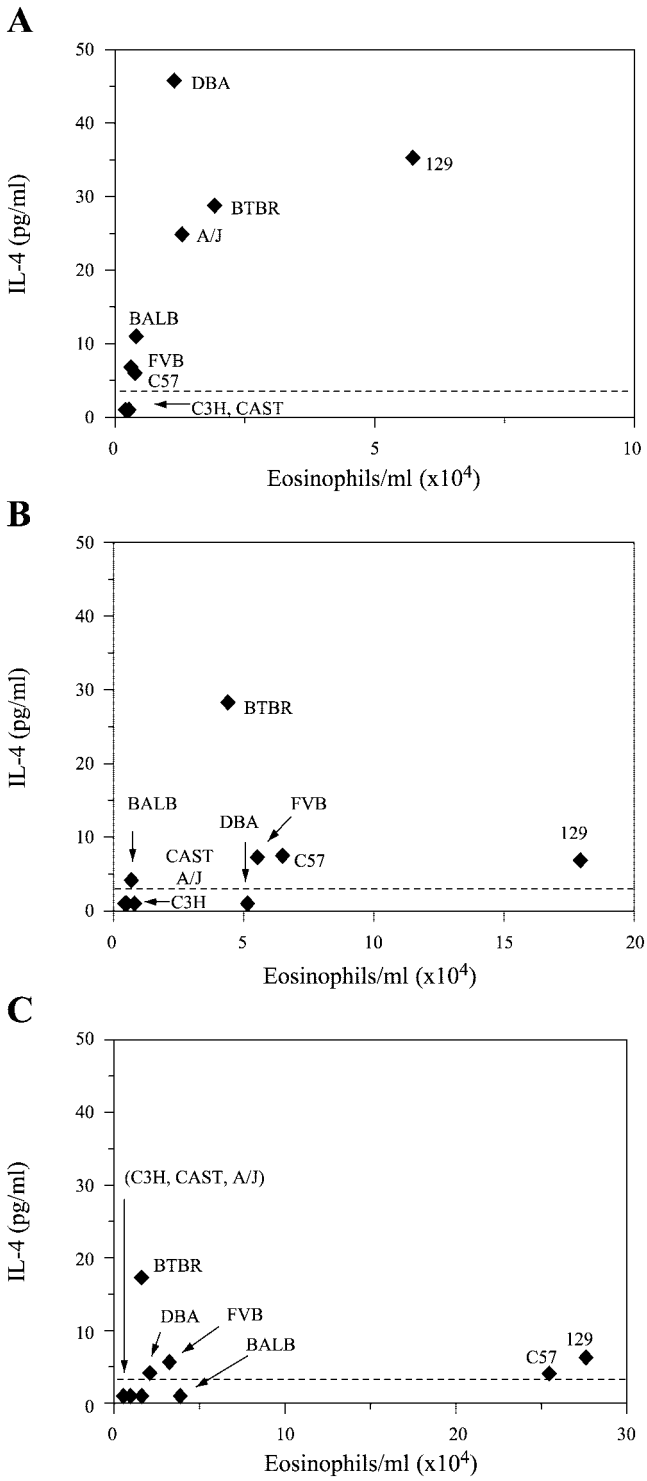

Fig. 4. Cosegregation plots for the mean number of eosinophils per milliliter recovered from whole lung lavage fluid of OVA mice vs. the expression levels of IL-4. The cosegregation plots were developed for data collected at 24 (*A*), 48 (*B*), and 72 (*C*) h postexposure. Note that the eosinophil scales (*x*-axis) are different for each time point. The dotted line represents the lower detection limit for IL-4. Data are presented as mean values for each strain.

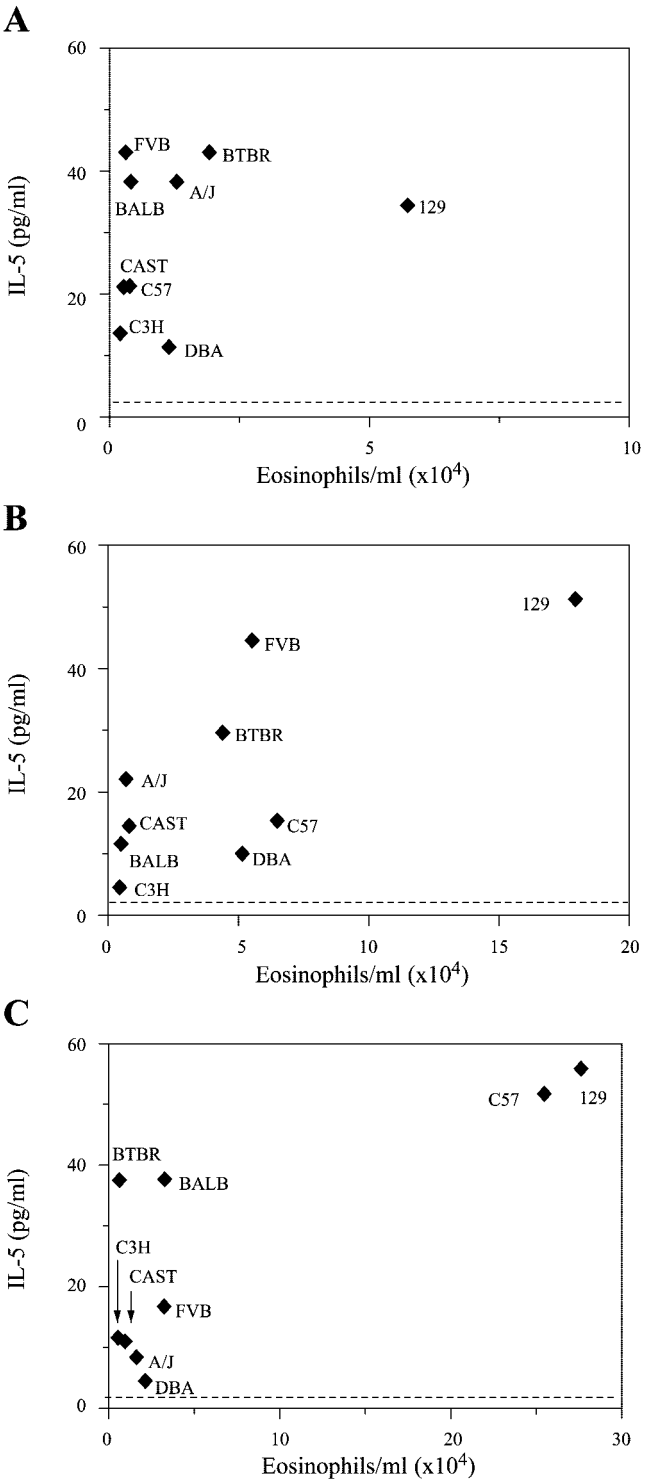

Fig. 5. Cosegregation plots for the mean number of eosinophils per milliliter recovered from whole lung lavage fluid of OVA mice vs. IL-5 expression levels. The cosegregation plots were developed for data collected at 24 (*A*), 48 (*B*), and 72 (*C*) h postexposure. Note that the eosinophils scales (*x*-axis) are different for each time point. The dotted line represents the lower detection limit for IL-5. Data are presented as mean values for each strain.

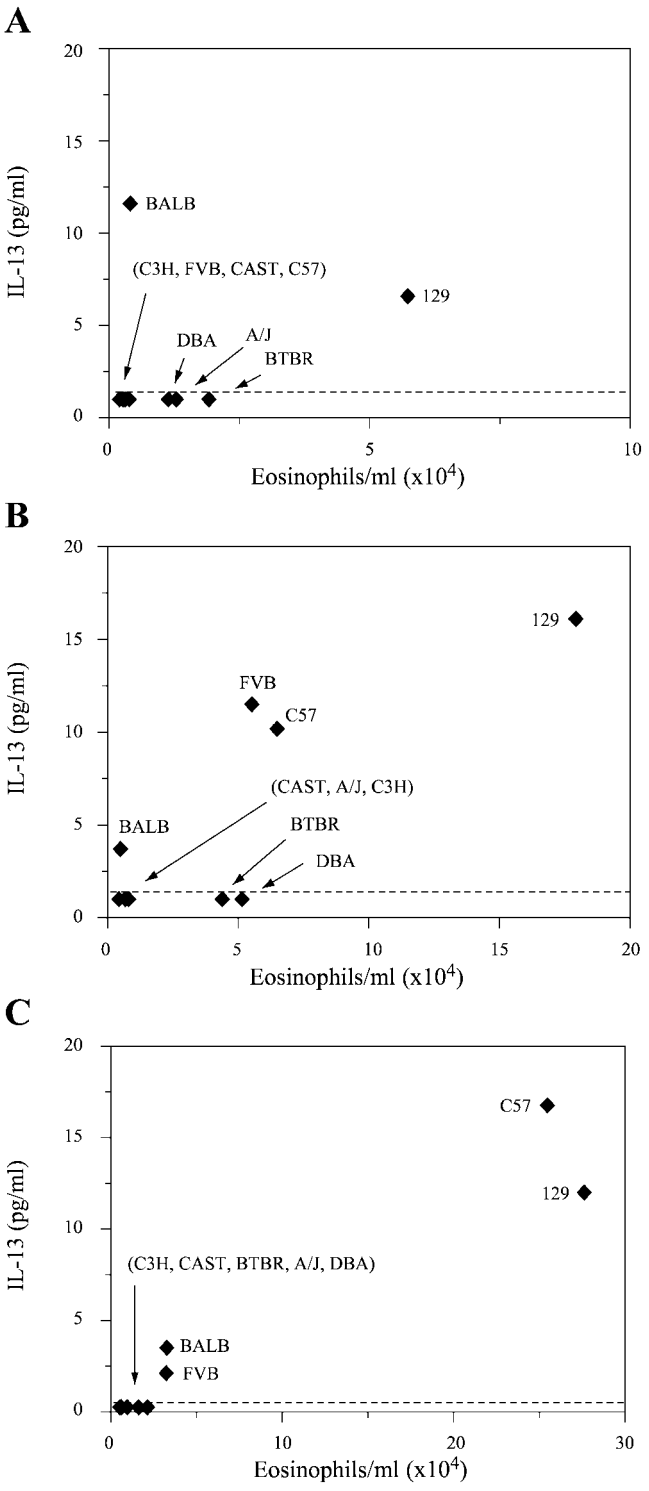

Fig. 6. Cosegregation plots for the mean number of eosinophils per milliliter recovered from whole lung lavage fluid of OVA mice vs. IL-13 expression levels. The cosegregation plots were developed for data collected at 24 (*A*), 48 (*B*), and 72 (*C*) h postexposure. Note that the scales for the *x*- and *y*-axes are different for each time point. The dotted line represents the lower detection limit for IL-13. Data are presented as mean values for each strain.

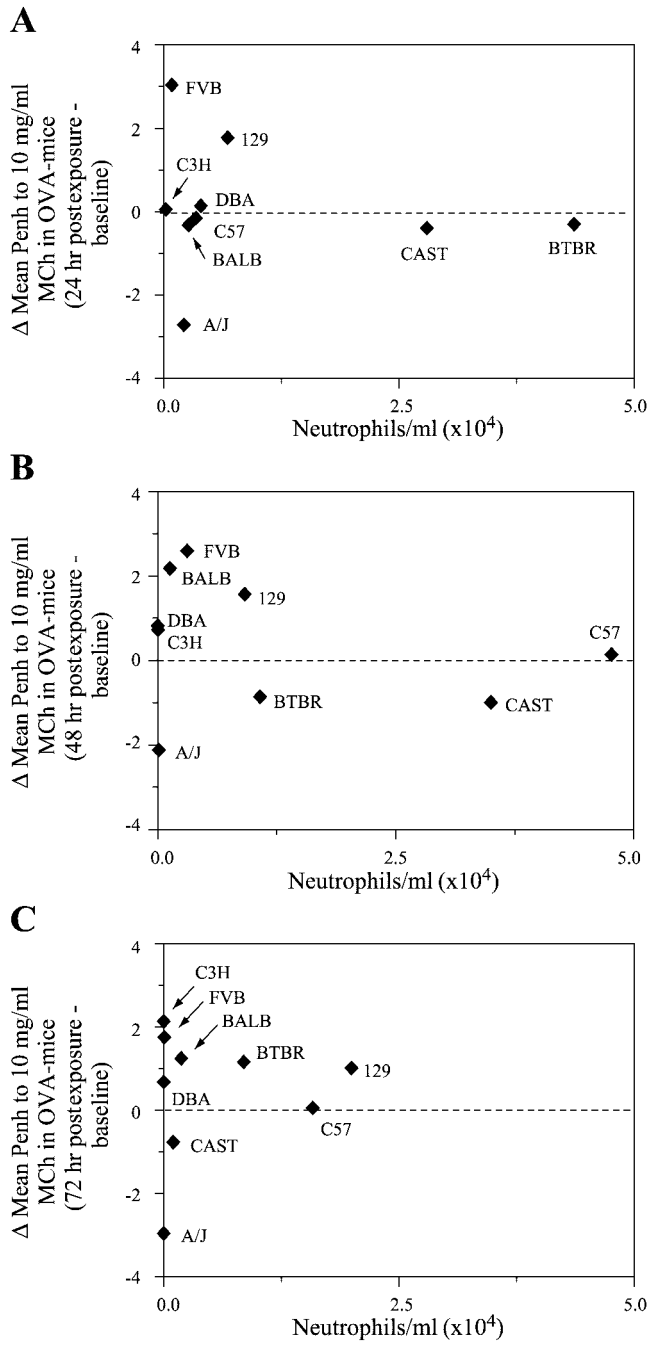

Fig. 7. Cosegregation plots for the mean Penh differences to MCh (10 mg/ml) challenge between post- and preexposure OVA mice vs. the mean number of neutrophils per milliliter recovered from whole lung lavage fluid of OVA mice. The cosegregation plots were developed for data collected at 24 (*A*), 48 (*B*), and 72 (*C*) h postexposure. Data are presented as mean values for each strain.

of allergen-induced airway disease. The mouse strains studied showed divergent temporal responses of AHR and airway inflammation after OVA sensitization and challenge. On the basis of the temporal relationships of allergen-induced AHR and airway inflammation, we were able to demonstrate a genetic component to the manifestation of allergen-induced airway disease.

AHR is usually a distinct feature of the airway response in an OVA-sensitized mouse model (14, 24). Whereas five strains (129/SvIm, BALB/cJ, C3H/HeJ, DBA/2J, FVB/NJ) displayed significantly increased airway responsiveness after OVA treatment, four strains [A/J, BTBR+(T)/tf/tf, CAST/Ei, and C57BL/6J] did not. These results are similar to those of other investigators who have shown that inbred mouse strains have variable AHR after an allergen sensitization and challenge (1, 40, 48). The current study also demonstrates temporal differences in the development of AHR to inhaled MCh among the strains of mice following the allergen challenge. These temporal differences in allergen-induced AHR may provide important phenotypic clues in identifying the genes controlling susceptibility to asthma.

Interestingly, our results differ from those of other investigations. For instance, other investigators found that the C57BL/6J strain develops AHR after sensitization and challenge to OVA (21, 40). In the present study, the C57BL/6J strain had only modest increases in AHR that were not statistically significant. The differences in the development of AHR of the C57BL/6J strain between these investigations and ours may have been due to mouse age or gender differences as well as the different protocols used. Furthermore, the differences may have been related to the method of determination of AHR to inhaled MCh. These other investigators measured resistance (R_L) and dynamic compliance (C_{dyn}) in anesthetized, tracheostomized mice to determine a change in airway function (21, 40). In the present investigation, we used changes in Penh as a measure of AHR. Although measures of Penh may not be as sensitive as the measurement of R_L and C_{dyn} to assess AHR, Penh measurements can be performed in unrestrained, unanesthetized mice and are well suited for assessment of AHR when mice need to survive (i.e., pre- and post-OVA exposure). Furthermore, our investigation agrees with previous studies that demonstrated that the C57BL/6J strain is one of the least responsive strains in the development of AHR to MCh following OVA sensitization and challenge (21, 40).

The cellular response appears to be central to the pathogenesis of allergen inflammatory airway disease. All of the nine strains sensitized and challenged to allergen had significant increases in leukocytes in the whole lung lavage fluid compared with the Alum-only-sensitized mice. The increases in leukocytes in the lavage fluid provide evidence of an inflammatory response to allergen sensitization and challenge in each of the mouse strains (39). In addition, we found that for all strains except the CAST/Ei strain, eosinophils made up the greater proportion of leukocytes present in the lung lumen. Interestingly, we did not find a consistent relationship between lung eosinophilia and AHR. For example, one of the strains with the highest levels of allergen-induced eosinophilia (C57BL/6J) did not develop AHR. This suggests that eosinophilia and AHR are independent of each other in this model. Several studies have also observed dissociation between eosinophilia and AHR in mice following allergen sensitization and challenge (10, 14, 16, 40, 41).

In agreement with previous studies that have also shown that inbred mouse strains exhibit different degrees of airway eosinophilia following allergen sensitization and challenge (1, 4, 15, 26, 40, 44, 48), the number of eosinophils was highest in the 129/SvIm and C57BL/6J strains and lowest in the C3H/HeJ, BTBR+(T)/tf/tf, and CAST/Ei strains. However, our results clearly demonstrate temporal differences in the infiltration of the eosinophils between genetically diverse inbred strains of mice. Several strains [BTBR+(T)/tf/tf, DBA/2J, and FVB/NJ] had peak infiltrates at 48 h postantigen exposure, whereas the remaining strains (129/SvIm, A/J, BALB/cJ, CAST/Ei, C3H/HeJ, and C57BL/6J) had peak infiltrates at 72 h postexposure. Because we found dissociations between eosinophilia and AHR in the mouse strains, the strains with different time courses of eosinophil infiltrates may also be excellent models to further pursue the genetics and biology involved in the development of this complex inflammatory lung disease.

Neutrophils were significantly elevated in the lung lavage of several strains (CAST/Ei, A/J, DBA/2J, C57BL/6J, and 129/SvIm) for at least one time point during the postexposure evaluation. Given that many investigations have shown neutrophils to be a major cellular component in allergen-induced asthma (2, 3, 9, 27, 36, 42) and that neutrophils are increased in humans with asthma (42), neutrophils may also be an important mediator in the inflammatory response following sensitization and exposure to an allergen. However, similar to our observations with eosinophils, we did not find a consistent relationship between lung neutrophilia and AHR. In addition, the LPS in the OVA exposures may have been responsible for increase of neutrophils present. All the mouse strains that had increased neutrophilia in our study have been found to respond to aerosolized LPS (29). Furthermore, the C3H/HeJ strain, which is unresponsive to LPS (29), did not have increased neutrophilia after OVA sensitization and challenge. Therefore, from these results, it is difficult to determine whether the increased airway neutrophilia was due to OVA sensitization and challenge or caused by small traces of LPS present in the aerosol OVA challenge.

Clinical and experimental investigations have indicated that a number of specific Th2 cytokines are important modulators of the inflammatory response in allergen-induced asthma (25, 34). IL-4, which is mainly produced by $CD4^+$ T cells, promotes the amplification of the Th2 inflammatory response (25, 34). It has been shown to induce the differentiation of naive $CD4^+$ T lymphocytes into Th2 cells. IL-4 also has other biological effects in the allergic response it shares with IL-13. Both IL-4 and IL-13 are believed to increase the expression of vascular cell adhesion molecule-1 on endothelial cells, which facilitates the recruitment of eosin-

ophils (11). In the present study, six of the nine strains of mice [BTBR+(T)/tf/tf, A/J, DBA/2J, BALB/cJ, C57BL/6J, and 129/SvIm] had significantly increased levels of IL-4 in the whole lung lavage fluid. The increased levels of IL-4 suggest that IL-4 may be an important mediator in the inflammatory response following sensitization and challenge to OVA (25). In addition, the elevated levels of IL-4 in the airways peaked in each of the six strains as early as 24 h postantigen, which preceded the maximum eosinophilic infiltration. The early expression of IL-4 suggests that its primary role occurs during the differentiation phase of the response, which has also been suggested by other investigators (43, 45). Furthermore, previous studies have demonstrated a correlation between increased levels of IL-4 and AHR (5). Although in the present study several strains (BALB/cJ, DBA/2J, and 129/SvIm) demonstrated good agreement between significantly increased levels of IL-4 and AHR, this relationship does not hold true for the C3H/HeJ and FVB/NJ strains. These strains demonstrated increased AHR with no significant increase in the levels of IL-4. This disassociation suggests AHR in mice can occur independently of IL-4, which is similar to the results found by Hogan et al. (16, 17).

IL-5, a cytokine known to regulate the growth, differentiation, activation, and survival of eosinophils (10, 28, 47), was significantly increased in the lavage fluid from seven of the nine strains after antigen challenge. In addition, IL-5 expression coincided with the infiltration of eosinophils for many of the strains. Elevated levels of IL-5 were present in the lung lavage for each strain and at each time point in which a minimum of 4.0×10^4 eosinophils/ml were counted in the lavage fluid. However, IL-5 levels in the lavage fluid were also increased at time points in which there were lower levels of eosinophil infiltrates. These results, similar to previous investigations, support a direct role for IL-5 in the development of airway eosinophilia in this model (10, 28, 47). Although many of the mouse strains had similarities of the time course of IL-5 levels in lavage fluid and eosinophilia, a significant correlation was not found. The potential role of IL-5 in the pathogenesis of allergen-induced airway disease remains unknown.

IL-13 was significantly increased in the whole lung lavage fluid in four of the nine strains (FVB/NJ, BALB/cJ, C57BL/6J, and 129/SvIm) after allergen sensitization and challenge. The increased expression of IL-13 occurred during the later phase of eosinophil infiltration at 48 and 72 h postexposure. Interestingly, the mouse strains in which IL-13 expression was increased were the strains with the highest number of lung eosinophil infiltrates during the later phase of eosinophilic infiltration. The increased expression and order of events suggest IL-13 may be linked to the inflammatory response in the lungs following allergen and sensitization.

In conclusion, our approach, based on an interstrain comparison of inbred mice, demonstrates that genetically diverse mice respond differently in the development of AHR and inflammation following OVA sensitization and challenge. The mouse strains show divergent temporal responses of AHR and inflammation in the lung airway. Several of the strains would be excellent models to further pursue the genetics and biology of allergen-induced airway disease.

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