

Exposure of Adult Mice to Environmental Tobacco Smoke Fails to Enhance the Immune Response to Inhaled Antigen

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Epidemiologic evidence supports a role for environmental tobacco smoke (ETS) in the occurrence and severity of allergies/asthma. However, neither the precise combination of ETS and allergen exposure nor the mechanism (or mechanisms) by which these factors interact and contribute to asthma induction is known. Animal model studies have failed to establish a convincing relationship between ETS exposure and asthma induction, perhaps because of methodological inadequacies. Here, we tested the hypothesis that ETS inhalation would provoke an asthmatic response by overcoming normal airway tolerance to inhaled antigens. Our protocol combined daily ETS exposure with nose-only sensitization to ovalbumin. Three strains of mice were tested, each with a different level of susceptibility to airway hypersensitivity. Immunological responses were assessed by immunoglobulin production. Airway inflammation was assessed by bronchoalveolar lavage differentials and lung histopathology. Airway hyperresponsiveness was determined by methacholine challenge. The mice produced ovalbumin-specific antibodies following ovalbumin exposure in a strain-dependent manner. Only the A/J mice produced detectable levels of ovalbumin-specific immunoglobulin (Ig) E. Both A/J and BALB/c mice produced ovalbumin-specific IgG1 antibodies. The C57Bl/6 mice did not produce detectable

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levels of antibodies. The A/J mice also exhibited airway inflammation following ovalbumin exposure. Neither the C57Bl/6 nor the BALB/c mice exhibited signs of airway inflammation. Exposure to ETS failed to enhance ovalbumin-specific antibody production, airway inflammation, or hyperresponsiveness. Together these results indicate that ETS exposure accompanied by nose-only allergen sensitization fails to overcome aerosol tolerance in adult mice.

Approximately 25% of children from developed countries have presented with wheezing in recent years, and half of these children later experience major asthma attacks (Lorente et al., 1998). This prevalence of asthma demands evaluation of potential risk factors to improve the possibility of prevention (Umetsu et al., 2002). However, the mechanisms involved in asthma induction remain unclear. The respiratory tract is routinely exposed to a variety of potentially allergenic substances without the induction of a hypersensitivity response (Murray et al., 2001; Braun-Fahrländer et al., 2002). The induction of T-cell tolerance likely protects most individuals from developing allergic airway diseases in response to commonly encountered allergens (Morel et al., 2003; Umetsu et al., 2003). In the case of atopic individuals, the underlying faults in regulating immune responsiveness appear to overcome this inherent safety mechanism (Izuhara et al., 2000; Seah et al., 2001). In nonatopic individuals, the process whereby aerosol tolerance is overcome remains unclear (Akbari et al., 2003).

External factors, including environmental pollutants, can affect the development of immune responses in the lung (Casillas & Nel, 1997; Sydbom et al., 2001; Eisner et al., 2002). While epidemiological studies have associated environmental tobacco smoke (ETS) exposure with increased incidence and severity of asthma (Gilliland et al., 2000; Mannino et al., 2001; Eisner, 2002), results of animal studies have been equivocal (Seymour et al., 1997; Barrett et al., 2002; Singh et al., 2003). Thus, in the one in vivo study where ETS exposure promoted a Th2 immune response to inhaled allergen (Seymour et al., 1997), responsive mice had previously been primed by intraperitoneal (ip) injections of allergen. Though effective at inducing an airway response, this protocol clearly fails to mimic the usual human situation of aerosol-only sensitization (Persson et al., 1997; Bice et al., 2000). Exposing mice to allergen exclusively via the airways and in the absence of alum [Al(OH)₃] can result in a reduced serum immunoglobulin (Ig) E response and a limited eosinophilic inflammatory response (Holt et al., 1981, 1987a; Seymour et al., 1998). This can be attributed to the induction of a form of immunological tolerance that specifically affects IgE production (Seymour et al., 1998; Clausen et al., 2003). In a study with transgenic mice that are predisposed to developing an allergic response to aerosolized ovalbumin (OVA), smoke exposure enhanced airway hyperreactivity (AHR) but was not associated with elevated lung eosinophilia or OVA-specific antibody production (Barrett et al., 2002). In a more recent study, adult mice presensitized by intratracheal instillation of *Aspergillus fumigatus* and then exposed to ETS displayed no enhancement of AHR (Singh et al.,

2003). Thus the role of ETS in altering immunological and physiological responses to allergens remains unclear (Gold, 2000).

We hypothesized that prolonged ETS exposure prior to or concurrent with nose-only OVA exposure of young adult mice would overcome aerosol tolerance, leading to an IgE antibody response and increased AHR. Further, we predicted that the pro-asthmatic responses would be most pronounced in those mice genetically predisposed to AHR. Thus, we exposed 3 strains of mice to 2 concentrations of ETS starting at 8 wk of age. Nose-only OVA priming was carried out at three different times during the ETS exposures. We assessed the effect of these co-exposures on the immunologic and pathophysiologic responses to the allergen.

METHODS

Animals

Three inbred strains of female mice were tested starting at 8 wk of age. Each strain reflects a different level of susceptibility to airway hypersensitivity: C57Bl/6 (resistant), BALB/c (moderately susceptible), and A/J (highly susceptible) (Fan et al., 1997; Wills-Karp & Ewart, 1997). The mice were obtained at 4–6 wk of age from Harlan Laboratories (Indianapolis, IN) or Jackson Laboratories (Bar Harbor, ME). Mice were housed and handled according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. All animal procedures were approved by the Louisiana State University Institutional Animal Care and Use Committee. Mice were on a 12-h light/dark cycle and were provided feed and water ad libitum, except when in the exposure chambers. Mice were housed in conventional plastic cages with corncob bedding (Anderson's, Inc., Maumee, OH) and transferred to wire-mesh cages for filtered air or ETS exposures.

ETS Exposure

ETS is composed of 85–90% sidestream smoke, with the remainder being exhaled mainstream smoke. Here we used sidestream smoke as a surrogate for ETS. ETS was generated by an AMESA 30-port cigarette smoking machine (AMESA Technologies, Geneva, Switzerland). All mainstream smoke was expelled from the smoke-generating chamber to a water trap. ETS was diluted with HEPA-filtered air to establish a targeted suspended particle load of 10 mg/m³. Smoke parameters were monitored continuously during exposures for carbon monoxide (CO) levels with a MIRAN saphiRE infrared spectrometer (Foxboro Co., Foxboro, MA) and by a particle

TABLE 1
ETS exposure parameters

	Target	Measured
Temp.	70°F	71 ± 1.5
RH	50%	53 ± 3.0
TSP	10 mg/m ³	10 ± 0.7
CO	45 ppm	44 ± 2.5

monitor (DustTrak, STI, St. Paul, MN) for total suspended particles (TSP). In addition, gravimetric analyses were performed every 2 h by weighing 0.45-mm membrane filters (Gelman, Ann Arbor, MI) through which measured amounts of smoke had been passed. Whole-body exposures to ETS or HEPA-filtered air (air controls) took place in a 1.3-m³ stainless steel and Plexiglas dynamic exposure chamber (5 h/day, 5 days/wk, 9 wk), starting when mice were 8 wk of age. Chamber airflow rate was maintained at 300 L/min (14 air changes/h). Targeted and measured ETS exposure parameters are given in Table 1.

Aerosol (Nose-Only) Sensitization

Mice were sensitized by nose-only aerosol exposure to phosphate-buffered saline (PBS) or 1% (w/v) OVA (Sigma grade VII) in PBS, in the absence of adjuvant, for a total of 10 days at various times during the ETS or air exposures (Table 2). Aerosols were generated by a nebulizer (Allegiance Healthcare, McGaw Park, IL) with a 7-L inhalation exposure chamber (In-Tox Products, Albuquerque, NM) and an airflow rate of 10 L/min. For nose-only exposures, mice were placed in plastic tubes designed to position the mouse nose in the aerosol flow stream while keeping the head and body free from antigen exposure. The tubes with the mice were connected to the 7-L chamber and exposed to 1% OVA in phosphate-buffered saline (PBS) for 20 min/day. Saline control mice were exposed similarly to PBS alone. For challenge exposures, mice were exposed to 5% OVA in PBS for 5 min. Total exposure per mouse was calculated using the values for tidal volume and breathing rate for a mouse at rest and for the amount of OVA deposited on a pre-weighed filter during the 20-min exposure period. The antigen exposure was estimated to be 70 µg/mouse.

OVA Priming

BALB/c and A/J mice were primed by intraperitoneal injections of OVA and alum starting at 8 wk of age. The primed mice

TABLE 2
Experimental time points (weeks of exposure)

	Exposure, air (A) or ETS (E)	Nose-only sensitization, saline (S) or OVA (O)	Necropsy
Early	1–9	2–3	12
Middle	1–9	5–6	15
Late	1–9	8–9	18

received 80 µg OVA in 4.5 mg alum on days 0 and 14, followed by nose-only aerosol sensitizations with 1% OVA (20 min/day) on days 30–32.

Pulmonary Function Testing

Airway function was assessed by whole body unrestrained plethysmography (Buxco, Troy, NY). Data are expressed as the “enhanced pause” (Penh) where $Penh = [(Te \text{ (expiratory time)} - Tr \text{ (relaxation time)})/Tr] \times [PEP \text{ (peak expiratory pressure in ml/s)}/PIP \text{ (peak inspiratory pressure in ml/s)}]$ and where Tr is the time of the pressure decay to 36% of total box pressure during expiration (Hamelmann et al., 1997b). Although there is some concern regarding the reliability of the Penh measurement for assessing changes in lung physiology (Lundblad et al., 2002), Penh remains a convenient indicator of alterations in airway mechanics (Hamelmann et al., 1997a; Chong et al., 1998). Mice were tested for AHR by methacholine (METH) challenge. Graded doses of nebulized METH (Sigma, St. Louis, MO) ranging from 1.56 mg/ml to 25 mg/ml were delivered by aerosol exposure. Baseline pulmonary function readings were obtained prior to METH exposure. Postexposure Penh values were averaged over 5 min.

BAL Sample Collection

Mice were euthanized with an intraperitoneal injection of 0.1 ml pentobarbital euthanasia solution. The chest was opened and blood was withdrawn by right ventricular cardiac puncture. The left ventricle was opened. The pulmonary circulation was flushed with approximately 2 ml of 37°C PBS by perfusion through the right ventricle. The right lung was clamped at the primary bronchus with a hemostat. The cervical trachea was exposed and then incised. A blunt cannula was inserted in the trachea and ligated in place. The left lung was lavaged 4 times with 0.3 ml of 37°C PBS. The first lavage sample was saved separately from the final three, which were pooled. The right lung was then removed and flash-frozen in liquid nitrogen. The left lung was perfused via the tracheal cannula with 0.3 ml of freshly prepared PLP fixative (0.02 M periodate, 0.1 M lysine, 0.25% paraformaldehyde in phosphate buffer, pH 7.4). The remainder of the lung was removed and immersed in PLP fixative and held 24–48 h before trimming.

Histopathologic Evaluation

Lungs were trimmed such that sections of the cranial, middle, and caudal lobes of the left lung were cut along the plane of each lobar bronchus. The samples were postfixed in 70% ethanol and imbedded in paraffin by routine procedure. Dehydration was carried out directly in graded alcohols to eliminate further exposure to formaldehyde. Sections were cut at 3–4 µm thickness and stained with hematoxylin and eosin. A 20-point, weighted scoring system was developed for histopathological evaluation of the lung sections. Seven categories were included: bronchus-associated lymphoid tissue (BALT), lymphocytes, plasma cells,

eosinophils, neutrophils, mucus metaplasia, and overall cellularity. The pathology scores are cumulative for the seven categories and represent the average of scores from two veterinary pathologists who were blinded to treatments.

Pulmonary Lavage Cytology

Cells from all lavage samples, pooled for each mouse, were sedimented by centrifugation at $300 \times g$ for 10 min. The cell pellet was resuspended in 0.5 ml PBS. A cytospin sample was prepared from approximately 6 drops of cell suspension and 1 drop of 22% bovine serum albumin and centrifuged at $80 \times g$ for 6 min in a Shandon Cytospin 3. The concentrated smears were stained with a modified Wright's stain and evaluated by a veterinary clinical pathologist. A 200-cell differential was obtained to determine the approximate percentages of neutrophils, macrophages, eosinophils, mast cells, and other cells.

Cytokine Quantitation in BAL Fluids

The BAL fluid samples were centrifuged and aliquots of the supernatants stored at -80°C until analyzed via a murine Th1/Th2 cytometric bead array (BD Pharmingen, San Diego, CA). This fluorescence-based detection system uses a sandwich immunoassay in which capture antibodies are immobilized on fluorescently-labeled microbeads. The samples to be assayed were added to the bead suspension and incubated for 2 h. Fluorescently labeled detection antibodies were added to the samples and incubated an additional 30 min. The samples were washed with FACS buffer and analyzed using a BD FACSCaliber. Standard curves were generated for each cytokine to allow for quantitative analyses.

Antibody Quantitation

Ovalbumin-specific IgE and IgG1 were quantitated from mouse sera by enzyme-linked immunosorbent assay (ELISA). Mouse blood was obtained either from the retro-orbital sinus or via cardiac puncture at sacrifice. Sera were stored at -70°C until assayed. Immunon-1B plates (Dynatech Laboratories; Chantilly, VA) were coated with $5 \mu\text{g}/\text{well}$ of OVA. Nonspecific binding was blocked with 2% teleostean gelatin (Sigma) in PBS prior to the addition of sera. Mouse sera was diluted 1/100 with PBS and added to each well. Antibody isotypes were detected with biotinylated isotype-specific antibodies (rat anti-mouse IgG1-biotin, clone A85-1; clone R19.5; rat anti-mouse IgE biotin, clone R35-118) followed by streptavidin conjugated with horseradish peroxidase (BD Bioscience, San Diego, CA). ELISA plates were developed with TMB peroxidase substrate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). Results are presented as OD_{405} readings for the samples.

Statistical Analysis

Each experimental group consisted of four to eight mice, and each experiment was performed twice with similar results. Treatment effects (ETS vs. air, OVA vs. saline) were analyzed

by a two-way repeated-measures analysis of variance (Sigmastat, SPSS, Chicago). Significance was determined at the $p < .05$ level. Post hoc analysis (Tukey's test) was used to identify interactions between treatment groups. Initial analysis of the data indicated no effect of timing of OVA exposure (early, middle, or late) on any of the measured parameters; therefore, for all subsequent analyses the data for each time point were combined for subsequent analysis of treatment effects.

RESULTS

Aerosol Exposure Induced OVA-Specific Antibodies in BALB/c and A/J Mice but Not in C57Bl/6 Mice; ETS Exposure Had No Effect on This Response

Three strains of mice were exposed to OVA via nose-only aerosolization to test whether this exposure method was sufficient to induce OVA-specific antibodies. While the C57Bl/6 mice failed to generate OVA-specific IgG1 antibodies (data not shown), the A/J and BALB/c mice produced significant amounts of OVA-specific IgG1 following nose-only sensitization (Figure 1; compare AS to AO).

To determine the effect of ETS exposure on the induction of OVA-specific antibodies, young adult female mice of each strain were exposed to ETS for 5 h/day for 9 wk and coexposed to

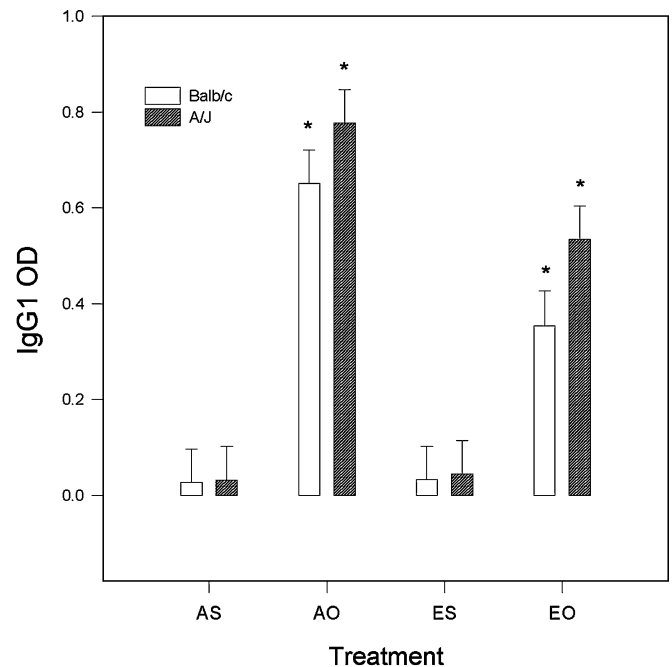


FIG. 1. Exposure to ETS fails to enhance the IgG1 antibody response to aerosolized OVA. BALB/c and A/J mice received 2 wk of nose-only exposure to either saline (S) or OVA (O) following whole-body exposure for 9 wk to ETS (E) or HEPA-filtered air (A). Results are presented as the average OD_{405} readings. Asterisk indicates significantly different from saline controls ($p < .01$, ANOVA).

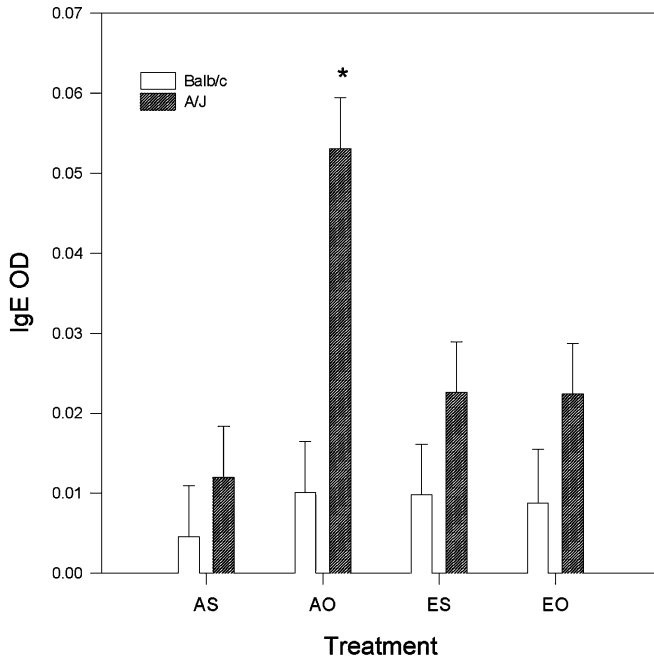


FIG. 2. Exposure to ETS fails to enhance the IgE antibody response to aerosolized OVA. BALB/c and A/J mice received 2 wk of nose-only exposure to either saline (S) or OVA (O) following whole-body exposure for 9 wk to ETS (E) or HEPA-filtered air (A). Results are presented as OD₄₀₅ readings. Asterisk indicates significantly different from saline controls ($p < .05$, ANOVA).

aerosolized OVA. Exposure to ETS reduced the IgG1 antibody response in both strains to that of the saline controls (Figure 1; compare EO to AO).

Only the A/J mice produced OVA-specific IgE antibodies following nose-only sensitization, although at very low levels and only in the air-exposed group (Figure 2; AS, AO). No ETS-exposed mice of any strain generated an IgE response to OVA (Figure 2; ES, EO). The OVA-primed A/J and BALB/c mice produced high levels of OVA-specific IgE ($OD_{405} > 2.0$).

BAL and Lung Histology

The predominant cell type present in all the BAL samples from the aerosol-exposed mice, regardless of treatment, was the alveolar macrophage (>98%). Minimal numbers (<1%) of eosinophils, mast cells, and other cells were observed. By contrast, the OVA-primed BALB/c mice exhibited elevated percentage of lymphocytes (24.3 ± 7.1) and eosinophils (17.3 ± 7.6) in their BAL fluids. OVA-primed A/J mice had even greater percentage of eosinophils (42.3 ± 4.4) and fewer lymphocytes (11.2 ± 1.5) in their BAL samples.

The A/J mice had mild to moderate inflammation in their lungs following OVA aerosol sensitization, whereas the effect was less pronounced in BALB/c mice; the AO pathology scores were significantly higher for the A/J than for BALB/c mice

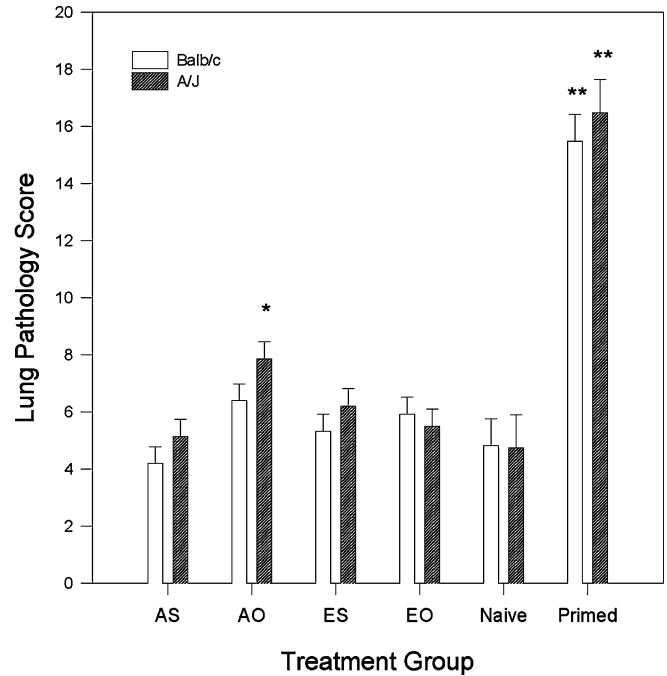


FIG. 3. Pathology scores of ETS- and OVA-exposed mice. A 20-point, weighted scoring system for bronchus-associated lymphoid tissue (BALT), lymphocytes, plasma cells, eosinophils, neutrophils, mucus metaplasia, and overall cellularity was used to evaluate hematoxylin and eosin (H&E)-stained lung sections. The scores represent the average from two pathologists independently reading the slides. Asterisk indicates significantly different from saline controls ($p < .05$, ANOVA); double asterisk, significantly different from all other groups ($p < .001$, ANOVA).

(Figure 3). Exposure to ETS and OVA (EO) failed to exacerbate the response in the BALB/c and reduced the inflammatory response in the A/J mice. C57Bl/6 mice exhibited no significant differences in pathology scores between treatment groups (data not shown). Intraperitoneal priming with OVA in alum adjuvant resulted in significant lung inflammatory responses in both the A/J and BALB/c mice.

Pulmonary Function

We used whole-body plethysmography to assess the effect of ETS exposure on AHR. The A/J mice were the most responsive of the three strains, with Penh values up to 5× higher than corresponding values in BALB/c mice (compare A/J responses in Figure 4, B and D, to the BALB/c responses in Figure 4, A and C). The increased Penh values in A/J mice appear to reflect a heightened sensitivity to inhaled METH, rather than to any of the test exposures, since there were no significant effects of OVA, ETS, or a combination of the two on airway responsiveness. Intraperitoneal priming of the mice led to only modest increases in the Penh responses (data not shown).

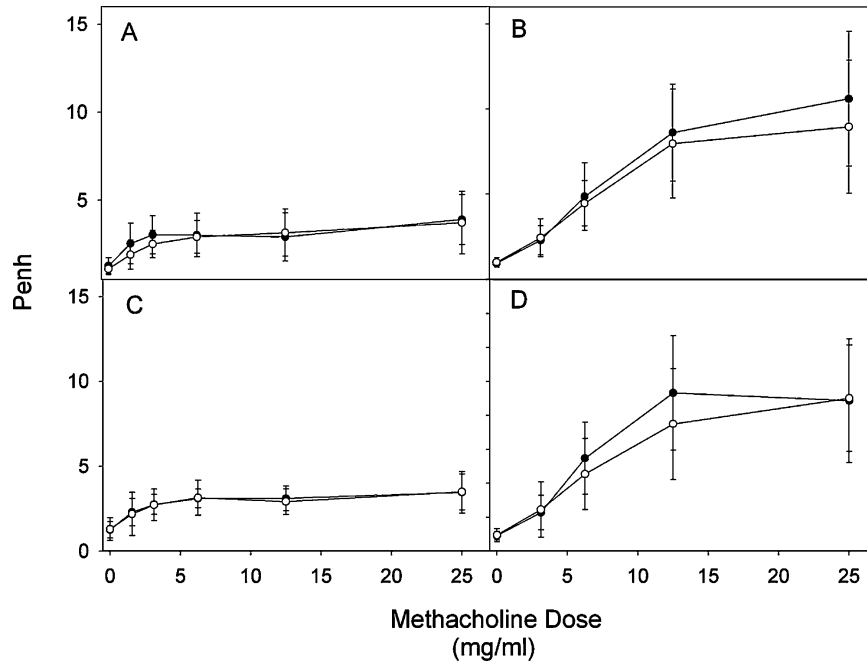


FIG. 4. Neither ETS nor aerosolized OVA exposure affected the airway response to inhaled methacholine. A/J (right panels) and BALB/c (left panels) mice were exposed to air (top) or ETS (bottom) prior to saline (open circle) or OVA (closed circle) nebulization. Airway function was assessed by whole-body unrestrained plethysmography. Graded doses of nebulized methacholine were delivered by aerosol exposure. Data are expressed as the “enhanced pause” (Penh).

BAL Cytokine

There was no consistent effect of either OVA or ETS exposure on cytokine levels in the BAL of the exposed mice (data not shown). The primed mice had significant ($p < .05$, ANOVA) amounts of IL-4 (9.633 ± 1.2 pg/ml) and IL-5 (224.7 ± 97.4 pg/ml) in their BAL fluids compared to saline control mice (<4 pg/ml).

DISCUSSION

The established murine model of allergic asthma requires both intraperitoneal and intranasal antigen administration for induction of maximal AHR and airspace eosinophilia (Zhang et al., 1997; Kannan & Deshpande, 2003). Development of the allergic response in this model is CD4+ T cell-dependent and is ameliorated by agents that either block Th2 cytokine action or prevent its induction (Gavett et al., 1995; Sur et al., 1996; Keane-Myers et al., 1997). Mice exhibit a strain-dependent susceptibility in this model, with a rank order of A/J > BALB/c > C57Bl/6 (Zhang et al., 1997; Morokata et al., 1999; Whitehead et al., 2003). While aerosol sensitization can be achieved (Renz et al., 1992), it generally is accepted that exposure to allergen exclusively via the airway leads to airway tolerance that is characterized by a reduced serum IgE and a limited inflammatory response (Holt et al., 1981; Holt & Leivers, 1982; Sedgwick & Holt, 1985; Holt et al., 1987a, 1987b; Seymour

et al., 1998). It has been proposed that airway tolerance is the mechanism whereby the development of allergic airway disease to inhaled antigens is prevented (Umetsu et al., 2003). The induction of airway allergies may be the result of environmental insults that overcome aerosol tolerance. Epidemiological studies have associated ETS exposure with increased incidence and severity of asthma (Gilliland et al., 2000; Mannino et al., 2001; Eisner, 2002). We hypothesized that ETS exposure would overcome aerosol tolerance, leading to an IgE antibody response and AHR in adult mice. Instead, we found that exposure to ETS prior to nose-only OVA sensitization failed to enhance either the histopathologic, pathophysiologic, or immunologic responses in any of the mice. Our AHR results are at odds with those obtained using transgenic mice predisposed to developing an allergic response to aerosolized OVA (Barrett et al., 2002). In that model, smoke exposure modestly enhanced AHR, though the authors used smoke and OVA levels that were higher than those employed in this study. Others have reported an inhibitory effect of short-term ETS exposure on AHR in mice (Melgert et al., 2004). In a recent study, mice exposed prenatally but not postnatally to cigarette smoke exhibited increased airway hyperresponsiveness to an *Aspergillus fumigatus* extract (Singh et al., 2003). Clearly, the effect of ETS on AHR in murine models is complex and dependent on both the duration and timing of the exposure. ETS exacerbation of AHR in humans is widely reported, though here again, differences in the degree of

susceptibility and severity also have been seen (Hemmelgarn & Ernst, 1997; Matsumoto et al., 1998; Belousova et al., 1999).

In contrast to the AHR results, our immunologic and pathophysiologic results are consistent with those obtained with transgenic mice, where ETS exposure was not associated with elevated lung eosinophilia or OVA-specific antibody production (Barrett et al., 2002). In the one in vivo study where ETS exposure promoted a Th2 immune response (Seymour et al., 1997) the mice were primed via an intraperitoneal injection of OVA in alum prior to ETS exposure. Thus, the mice already had already mounted a Th2 immune response and the exposure to ETS exacerbated this ongoing response. In our study, nose-only exposure alone fails to induce an initial Th2 immune response in the BALB/c mice and ETS exposure did little to enhance this response. Even in the A/J mice, which likely mounted a weak Th2 response as evidenced by the production of low levels of OVA-specific IgE antibodies, ETS exposure failed to enhance this response. As such, our results indicate that ETS exposure alone is insufficient to overcome aerosol tolerance in adult mice.

Unlike the case for ETS, the contribution of diesel exhaust particulates (DEP) to the increased incidence of allergies and asthma (Peterson & Saxon, 1996; Davies et al., 1998) has been supported by animal model studies (Nel et al., 1998; Fujimaki et al., 2001; Pandya et al., 2002; Finkelman et al., 2004). Likewise, exposure of young mice to residual oil fly ash (ROFA) and aerosolized OVA has been reported to overcome aerosol tolerance (Hamada et al., 2000). This has not been the case in regard to ETS as shown here and elsewhere (Barrett et al., 2002; Singh et al., 2003). While differences in the molecular composition of ETS and the other pollutants likely account for the varying biological effects, other reasons for this disparity could include any of the following: (1) Unlike the situation for heart disease and cancer, ETS does not play a causative role in the increased prevalence of asthmatic disease. (2) ETS may play a role in asthma induction in humans, but not in mice because the mechanism for overcoming aerosol tolerance differs in the two species. (3) ETS may be inhibiting rather than promoting immune responses to the antigen. There have been numerous reports on the suppressive effect of mainstream smoke and cigarette smoke condensate on immune function (Jacob et al., 1980; Petersen et al., 1983; Johnson et al., 1990). Chronic (>21 wk) exposure to secondhand exposure has been reported to inhibit antibody responses in rats (Sopori et al., 1989). However, other studies using shorter exposures failed to identify an immunosuppressive effect (Seymour et al., 1997; Barrett & Bice, 2001). Nevertheless, the possibility of ETS exposure modifying the immune response in some manner other than overcoming aerosol tolerance cannot be discounted since we did observe a decrease in IgG1 antibody responses in the ETS-exposed mice. (4) ETS exposure of adults is inadequate to overcome aerosol tolerance. In support of the last point, there is increasing suspicion that fetal exposure to allergens (and pollutants) including ETS may contribute to the increase in asthmatic diseases in adults (Vance & Holloway, 2002). Additional studies examin-

ing the effect of fetal exposure to ETS on aerosol tolerance are warranted.

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