Allergen-Independent Maternal Transmission of Asthma Susceptibility

Kaoru Hamada, Yasue Suzaki, Alejandra Goldman, Yao Yu Ning, Carroll Goldsmith, Aiyappa Palecanda, Brent Coull, Cedric Hubeau and Lester Kobzik

*J Immunol* 2003; 170:1683-1689;
doi: 10.4049/jimmunol.170.4.1683
http://www.jimmunol.org/content/170/4/1683
Allergen-Independent Maternal Transmission of Asthma Susceptibility

Kaoru Hamada, Yasue Suzaki, Alejandra Goldman, Yao Yu Ning, Carroll Goldsmith, Aiyappa Palecanda, Brent Coull, Cedric Hubeau, and Lester Kobzik

Maternal asthma is a risk factor for development of asthma in children, but mechanisms remain unclear. Offspring of asthmatic mother mice (sensitized and repeatedly exposed to OVA Ag) showed airway hyperresponsiveness and allergic pulmonary inflammation after an intentionally suboptimal OVA sensitization and exposure protocol that had little effect on normal offspring. Similar results were obtained when offspring of OVA-allergic mothers were exposed to an unrelated allergen, casein, indicating that the maternal effect is allergen independent and not transferred by OVA-specific Abs. Premating treatment with neutralizing anti-IL-4 Ab or reduction of maternal allergen exposure abrogated the maternal effect, showing a critical mechanistic role for IL-4 and suggesting an additional benefit of allergen avoidance.


The origins of asthma in early life (1) suggest that prenatal events may influence susceptibility to allergic airway disease. Indeed, epidemiologic studies report the intriguing finding that maternal, more than paternal, asthma is a significant risk factor for development of asthma (2–4). This finding has prompted speculation about potential pathways for maternal influence on the fetal immune system, including transfer of mediator(s) from mother to child (e.g., allergens (5, 6), Abs (7), or other immune mediators via the placenta (8, 9)), which might prime or sensitize the developing individual for increased susceptibility to asthma (10, 11). These postulates reflect experimental data demonstrating newborn immune responses to maternal vaccines or Ags in the maternal environment (5, 12, 13), but are somewhat in conflict with earlier observations that transfer of maternal Ag-specific IgG may protect against allergy by suppressing neonatal IgE responses (14). Progress in this area has been hampered by the lack of an experimental model and the complex epidemiology of asthma risk factors in people, including roles for genetic (15) and environmental (e.g., maternal smoking (16)) factors. We reasoned that these obstacles to identifying specific mechanisms for maternal immune effects could be overcome in a murine model of asthma that uses inbred mice with identical genetic and environmental backgrounds.

The studies used female mice sensitized as neonates and exposed repeatedly to aerosolized OVA Ag. This results in allergen-induced airway hyperresponsiveness (AHR), pulmonary allergic inflammation (AI), and serum OVA-specific IgE, which are features that recall human asthma in this commonly used model (17, 18). After mating, we compared offspring of normal or asthmatic mother mice for susceptibility to development of the asthma phenotype (AHR and AI) upon exposure to allergen (OVA) in early life. Our strategy was to use an intentionally suboptimal protocol in which only a single i.p. injection was used for sensitization, rather than the two injections that consistently give robust AHR and AI when animals are subsequently challenged with aerosolized allergen (19, 20). After observing increased susceptibility in this basic model, we investigated whether or not the maternal effect is allergen specific by challenge of baby mice with a second, unrelated allergen, casein (Cs). We also identified a critical role for active allergic inflammation in the asthmatic mother mouse by manipulation of premating allergen exposures and by premating treatment of mothers with neutralizing anti-IL-4 Ab.

Materials and Methods

Animals

Newborn BALB/c mice were obtained commercially from Harlan Sprague Dawley (Indianapolis, IN) as litters with their mother mouse at day 2 of age or by in-house breeding, as described below. Each mother and litter was housed separately, fed a commercial pelleted mouse feed, and given water ad libitum. The mice were housed in an animal facility that was maintained at 22–24°C with a 12-h dark/light cycle. All experimentation was conducted under a protocol approved by our institutional review board. All reagents not otherwise specified were obtained from Sigma-Aldrich (St. Louis, MO).

Allergen sensitization and exposure

Maternal sensitization was achieved by initial i.p. injections of 0.1 ml PBS containing OVA (5 μg) and alum (1 mg) into mice at 3 and 7 days of age. After weaning, female mice were exposed to aerosols of allergen (3% w/v OVA (grade III, Sigma-Aldrich) in PBS, pH 7.4) for 10 min on 3 consecutive days at 4, 8, and 12 wk of age. The aerosol exposure was performed within individual compartments of a mouse pie chamber (Braintree Scientific, Braintree, MA) using a Pari SST nebulizer (Sun Medical Supply, Kansas City, KS) connected to air compressor (PulmoAID; DeVilbiss, Somerset, PA) (21). Immediately after the last aerosol exposure, the female mice were placed in cages with male mice to allow mating. At ~ day 18 of pregnancy, some mice were further exposed to an aerosol challenge of OVA for each of 3 consecutive days, as above. After birth, baby mice were treated with a single i.p. injection of OVA and alum on day 3. On days 12–14 of life, these baby mice were exposed to aerosolized OVA, as above. Physiologic and pathologic analysis was performed the next day (age day...
The experimental protocol is summarized in Fig. 1. In some experiments, female mice were injected with rat anti-mouse IL-4 Ab (1 mg i.p., clone 11B.11; National Cancer Institute, Frederick, MD). Controls for the four major variables of the protocol included omission of treatment for the first three (maternal sensitization, OVA aerosol exposure of pregnant mice, newborn in utero sensitization) and substitution of PBS for OVA in the final aerosol exposure of newborns. Treatment groups are coded by their exposures in these four stages, using O for OVA, P for PBS and — for no treatment, as summarized in Table I. Similar protocols were used for studies with a second allergen, Cs, with the modification that aerosol challenge with Cs was performed using a 1% solution for 20 min.

### Pulmonary function testing

Airway responsiveness of mice to increasing concentrations of aerosolized methacholine was measured using whole body plethysmography (Buxco, Sharon, CT). Briefly, each mouse was placed in a chamber, and continuous measurements of airway pressure/time wave were calculated via a connected transducer and associated computer data acquisition system. The main indicator of airflow obstruction, enhanced pause (Penh), which shows strong correlation with the airway resistance examined by standard evaluation methods, was calculated from the box waveform (22). After measurement of baseline Penh, aerosolized PBS or methacholine in increasing concentrations (6, 12, 25, 50, and 100 mg/ml) was nebulized through an inlet of the chamber for 1 min, and Penh measurements were taken for 9 min after each dose. Penh values for the first 5 min after each nebulization were averaged and used to compare results across treatment groups and individual mice.

### Pathologic analysis

After physiologic testing in airway-sensitized mice or postallergen-challenged mice, the animals were euthanized with sodium pentobarbital (Ventricular Laboratories, Lenexa, KS). The chest wall was opened and the animals were exsanguinated by cardiac puncture. Serum was prepared and stored at −80°C. After lavage, the lungs were instilled with 10% buffered Formalin, retrieved, and fixed in the same solution. After paraffin embedding, sections for microscopy were stained with H&E. An index of pathologic changes in bronchoalveolar lavage (BAL) was performed five times with 0.3 ml (after allergen challenge) of sterile PBS instilled and harvested gently. Lavage fluid (recovery volume was ~90% of instilled) was collected and centrifuged at 1200 rpm (300 × g) for 10 min, and the cell pellet was resuspended in 0.5 ml PBS. Total cell yield was quantified by hemocytometer. BAL differential cell counts were performed on cytocentrifuge slides prepared by centrifugation of samples at 800 rpm for 5 min (Cytospin 2; Shandon, Pittsburgh, PA). These slides were fixed in 95% ethanol and stained with Diff-Quick (VWR, Boston, MA), a modified Wright-Giemsa stain, and a total of 200 cells were counted for each sample by microscopy. Macrophages, lymphocytes, neutrophils, and eosinophils were enumerated.

After lavage, the lungs were instilled with 10% buffered Formalin, removed, and fixed in the same solution. After paraffin embedding, sections for microscopy were stained with H&E. An index of pathologic changes in coded H&E slides was derived by scoring the inflammatory cell infiltrates around airways and vessels for greatest severity (0, normal; 1, ≤3 cell diameter thick; 2, 4–10 cells thick; 3, ≥10 cells thick) and overall extent (0, normal; 1, <25% of sample; 2, 25–50%; 3, 51–75%; 4, ≥75%). The index was calculated by multiplying severity by extent, with a maximum possible score of 9. The extent of goblet cell hyperplasia in airway epithelium was scored on a similar 0–3 scale.

### Assay of serum Ig

Anti-OVA-specific IgE Ab was measured by ELISA (19). Ninety-six-well microtiter plates (Nunc, VWR, Boston, MA) were coated with 0.2 μg of monoclonal anti-mouse IgE (BD Pharmingen, San Diego, CA) diluted in 0.1 M carbonate buffer (pH 9.5). After overnight incubation at room temperature, plates were washed with PBS-0.05% Tween and blocked with PBS-BSA (5% w/v, pH 7.4) for 1 h, followed by addition of serum samples. After overnight incubation at room temperature and washing, biotinylated OVA (1 μg/ml) was added to the plates. Plates were incubated for 1 h at room temperature and washed. After another 1-h incubation with streptavidin-HRP (Zymed, San Francisco, CA; 1:4000), the reaction was developed with tris(3,5-dimethylbenzidine) substrate (TMB One-Step; Dako, Carpenteria, CA). Plates were read in a Softmax plate reader (Molecular Devices, Menlo Park, CA) at 450 nm. For a positive control standard, we used a purified, monoclonal anti-OVA IgE developed using standard hybridoma generation techniques, as previously described (23), and the spleenocytes of a BALB/c mouse injected repeatedly with OVA and alum. After screening for anti-OVA binding using direct ELISA, we identified a clone (2C6) secreting an IgE anti-OVA (characterization detailed in Fig. 3), which was purified and used to standardize subsequent measurements of serum anti-OVA IgE. The ELISA above can detect concentrations of ≥50 ng/ml of this Ab when used as a standard control. Serum concentrations of rat IgG were measured using a standard sandwich ELISA method with a standard curve provided by serial dilutions of the rat IgG anti-IL-4 (clone 11B.11 (24)).

### Statistical analysis

Data are presented as mean ± SE. ANOVA analysis of differences among group means was performed using Fisher’s protected least significant difference test and the Statview software program (Abacus Concepts, Berkeley, CA). Statistical significance was accepted when *p ≤ 0.05.

### Table I. Coding of experimental groups

<table>
<thead>
<tr>
<th>Code</th>
<th>Maternal Allergy</th>
<th>Pregnancy Aerosol Exposure</th>
<th>Neonatal Sensitization</th>
<th>Aerosol Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>O/O/O/O</td>
<td>OVA</td>
<td>OVA</td>
<td>OVA</td>
<td>OVA</td>
</tr>
<tr>
<td>O/−−O/O</td>
<td>OVA</td>
<td>OVA</td>
<td>OVA</td>
<td>OVA</td>
</tr>
<tr>
<td>−/−/O/O</td>
<td>−</td>
<td>OVA</td>
<td>−</td>
<td>OVA</td>
</tr>
<tr>
<td>O/−/−/P</td>
<td>OVA</td>
<td>OVA</td>
<td>−</td>
<td>PBS</td>
</tr>
<tr>
<td>O/−−/−/</td>
<td>OVA</td>
<td>OVA</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>−/−/−/−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>O/Cs/Cs</td>
<td>OVA</td>
<td>Cs</td>
<td>Cs</td>
<td>Cs</td>
</tr>
<tr>
<td>−/−−/Cs/Cs</td>
<td>−</td>
<td>Cs</td>
<td>−</td>
<td>Cs</td>
</tr>
</tbody>
</table>

* A summary of the coding scheme used to label different experimental groups is shown (O, OVA; P, PBS; −, no treatment).
exposed mothers. The efficacy of the protocol in creating allergic airway disease in the female (future mother) mice was evaluated after each of the aerosolized allergen challenges at 4, 8, and 12 wk of age. The results showed AHR and AI after allergen exposure (Fig. 2). We subjected the offspring obtained by mating of asthmatic or normal mice to an intentionally suboptimal sensitization with OVA (a single i.p. injection) before aerosolized allergen challenge on days 12–14 of life. The experimental design results in four major variables that define the status of the young mice at the end of the protocol. These variables and the coding system used to designate them are summarized in Table I.

Results
Susceptibility to OVA-specific allergic airways disease

The basic protocol for these studies (summarized in Fig. 1) was performed on mice derived from either normal or OVA-sensitized/ exposed mothers. The efficacy of the protocol in creating allergic

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Inflammation Index</th>
<th>Goblet Cell Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>O/O/O/O</td>
<td>42</td>
<td>2.4 ± 0.3*</td>
<td>1.4 ± 0.1*</td>
</tr>
<tr>
<td>O/−/−/O</td>
<td>29</td>
<td>2.4 ± 0.4*</td>
<td>1.7 ± 0.2*</td>
</tr>
<tr>
<td>−/−/−/O</td>
<td>44</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>O/O/−/P</td>
<td>12</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>−/−/−/P</td>
<td>8</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>O/O/Cs/Cs</td>
<td>12</td>
<td>2.0 ± 0.6*</td>
<td>1.7 ± 0.3*</td>
</tr>
<tr>
<td>O/−/Cs/Cs</td>
<td>4</td>
<td>2.0 ± 0.6*</td>
<td>2.5 ± 0.9*</td>
</tr>
<tr>
<td>−/−/Cs/Cs</td>
<td>14</td>
<td>1.1 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
</tbody>
</table>

* An index of pathologic changes in coded H&E slides was derived by scoring the inflammatory cell infiltrates around airways and vessels for greatest severity (0, normal; 1, ≤3% cell diameter thick; 2, 4–10 cells thick; 3, ≥10 cells thick) and overall extent (0, normal; 1, <25% of sample; 2, 25–50%; 3, 51–75%; 4, ≥75%). The index was calculated by multiplying severity by extent, with a maximum possible score of 9. The extent of goblet cell hyperplasia in airway epithelium was scored on a similar 0–3 scale.

†, p < 0.01 vs −/−/−/O, O/O/−/P, −/−/−/P groups.

‡, p < 0.01 vs −/−/Cs/Cs, −/−/−/P groups, ANOVA; similar results obtained using nonparametric analysis (data not shown).

FIGURE 3. Maternal allergy promotes development of asthma phenotype in baby mice. A. Airway responsiveness (Penh) was significantly increased in offspring of OVA-sensitized and exposed mothers, with or without allergen exposure during pregnancy (●, O/O/O/O; ■, O/−/−/O), over that seen in offspring of normal mothers (○, −/−/−/O) as well as other controls (□, O/O/−/−O; □, O/O/−/−P; ○, −/−/−/−/O). *, **, p < 0.01 vs all other groups at 12–100 mg/ml methacholine, n ≥ 16, each group; B, offspring of OVA-sensitized and exposed mother mice subjected to OVA sensitization and aerosol exposure showed increased eosinophils (×105) in BAL samples (O/O/O/O, O/−/−/O; *, †, p < 0.01 vs controls, n ≥ 16). Histopathology showed accumulation of lymphocytes and eosinophils around airways and vessels and goblet cell hyperplasia (C, O/O/O/O; D, O/−/−/−/O, ×200). Offspring of normal mother mice subjected to the same OVA sensitization and aerosol exposure (E, −/−/−/−/O) manifest a lesser, albeit detectable, degree of pathologic change compared with normal lung seen in normal controls exposed only to (PBS) aerosols (F, −/−/−/−/P). See also scoring of changes in Table II.

FIGURE 4. Evaluation of serum anti-OVA IgE. The specificity of the purified anti-OVA IgE mAb (clone 2C6) used to standardize ELISA of sera samples is illustrated in A, which shows reactivity in direct ELISA with OVA, but not with other control proteins. A typical standard curve in our ELISA using increasing amounts of 2C6 to capture a constant amount of biotinylated OVA (10 μg/ml) is illustrated in B, which also shows minimal background in this assay from an irrelevant (antitrinitrophenyl) control mouse IgE (mIgE). C, OVA-specific IgE levels were elevated in offspring of OVA-sensitized and exposed mother mice, including those subjected to OVA sensitization and aerosol exposures (O/O/O/O, O/−/−/−/O) and those sensitized and exposed only to PBS (O/−/−/−/P), *, p < 0.01 vs offspring of normal mothers (−/−/−/−/O/O, −/−/−/−/−/P, ND = not detected; n ≥ 10).
Babies from asthmatic, but not normal, mother mice showed: 1) airway hyperresponsiveness to methacholine (increased Penh; Fig. 3A); 2) increased eosinophils on BAL (Fig. 3B); and 3) robust pathologic changes of AI (eosinophil and mononuclear cell infiltration around airway and vessels and goblet cell hyperplasia) (Fig. 3, C and D). Results of semiquantitative scoring of histology support the qualitative changes illustrated in Fig. 3, C–F, and are presented in Table II. It is noteworthy that the maternal effect was seen whether or not OVA-allergic mothers were subjected to an additional OVA aerosol challenge during pregnancy (i.e., O/O/O/O vs O/O/O/O; Fig. 3). Treatment groups in which sequential components of the sensitization protocol were omitted or replaced with control PBS were tested and mostly showed minimal airway responsiveness and AI.

Allergen-specific IgE

To investigate the contribution of allergen-specific IgE, we measured serum anti-OVA IgE. The characterization of a purified anti-OVA IgE mAb (2C6) developed and used as a reference reagent for the standard curves in these ELISA is presented in Fig. 4, A and B. Serum OVA-specific IgE was increased in offspring of asthmatic mothers (Fig. 4C), including unsensitized offspring that did not show AHR or AI (e.g., the O/O/−/P group; see Figs. 3 and 4). Similarly, elevated levels of OVA-specific IgG were detected in asthmatic mothers, their offspring, and breast milk from asthmatic mothers (data not shown). These data and the similar absence of AHR and AI in unsensitized offspring exposed to OVA allergen aerosols (O/O/−/O) indicate that allergen-specific Ab is transferred from mother to offspring in this model, but is not sufficient to confer sensitization and allow development of AHR and AI upon challenge with aerosolized allergen. This indicated that the maternal effect might be allergen independent and represents a more generalized increase in allergic susceptibility, as suggested by the increased immediate contact hypersensitivity to a second allergen observed in offspring of OVA-allergic female mice (25, 26).

Susceptibility to respiratory allergy to a different allergen

To more directly test the role of allergen and/or Ab in maternal transfer of susceptibility, we replaced the OVA allergen used for sensitization and challenge of baby mice with Cs. This was based on development of a mouse model of asthma using this second,

![Figure 5](http://www.jimmunol.org/)

**FIGURE 5.** Allergen independence of maternal effect. A. Airway hyperresponsiveness is seen in BALB/c mice sensitized by two i.p. injections of Cs at 3 and 7 days of age, and exposed to aerosols of Cs on days 12–14 of age (■) relative to similarly sensitized mice exposed to PBS aerosols (●). *, *p < 0.01 at 12–100 mg/ml methacholine, n ≥ 12. B. Minimal airway responsiveness is seen in offspring subjected to sensitization with a single i.p. injection of allergen (Cs) and subsequent Cs aerosol challenge as above (□, −/−/Cs/Cs). In contrast, offspring of OVA-sensitized and exposed mother mice subjected to this Cs sensitization and challenge protocol showed airway hyperresponsiveness (with or without OVA allergen exposure during pregnancy; ■, O/O/Cs/Cs; ○, O/O/−/Cs/Cs; *, *p < 0.01 vs −/−/Cs/Cs at 12–100 mg/ml methacholine) and eosinophilia (× 105) upon BAL analysis (C, *, †, *p < 0.01 vs −/−/Cs/Cs controls; n ≥ 16). Histopathology showed allergic airway inflammation and vessels and goblet cell hyperplasia in (D) normal mice sensitized with two i.p. injections of Cs and subsequently exposed to aerosolized CS and (E, F) offspring of OVA-sensitized and exposed mother mice subjected to a single Cs sensitization and challenge (O/O/Cs/Cs, O/O/−/Cas/Cas, respectively), but was unremarkable in offspring of normal mice similarly treated (G, −/−/Cs/Cs).
distinct protein Ag (bovine Cs). Analysis showed marked AHR and AI in baby mice that were sensitized with two i.p. injections of Cs with alum adjuvant before challenge with aerosolized Cs allergen, with minimal response to aerosolized PBS (Fig. 5, A and D). Similar results were found in adult mice (results not shown), confirming that Cs could be used like OVA to create mouse models of allergic airway disease. We modified the previous protocol (as outlined in Fig. 1) to test the response of babies born to OVA-allergic and exposed mothers to sensitization with a single i.p. injection of Cs, followed by challenge with Cs aerosols (days 12–14) and evaluation (day 15). Babies from OVA-asthmatic, but not normal, mother mice showed marked susceptibility to sensitization by the single i.p. treatment with Cs. This was manifest as: 1) AHR to methacholine (increased Penh; Fig. 5B); 2) increased eosinophils on lung lavage (Fig. 5C); and 3) robust pathologic changes of AI (Fig. 5, E and F). Results of semiquantitative scoring of histology support the qualitative changes illustrated in Fig. 5 and are presented in Table II. As with OVA, in these experiments using a second Ag, the maternal effect was seen whether or not OVA-allergic mothers were subjected to an additional OVA aerosol challenge during pregnancy (i.e., O/O/Cs/Cs vs O/−/Cs/Cs; Fig. 5).

Maternal mechanisms

Increased susceptibility to a second, unrelated Ag indicates that transfer of allergen or allergen-specific Abs does not mediate the maternal influence observed. The data suggest instead transfer of allergen-independent mediators that prime or enhance an asthma-like immune response of offspring. Notably, re-exposure to allergen during pregnancy was not required for increased susceptibility to either OVA or Cs. We postulated that cytokine(s) induced during the active allergic inflammation produced in the (future) mother mice just before mating might persist and act on the developing immune system of their offspring during pregnancy. We focused these initial studies on IL-4, a cytokine well characterized for its role in allergic airway inflammation (27, 28).

To test these postulates, we performed two related experiments. In the first, we omitted the final premating OVA aerosol challenge of sensitized female mice (as well as omitting any allergen exposure during pregnancy). Offspring of mice treated this way no longer showed susceptibility to sensitization to either OVA or Cs, with no AHR (Fig. 6, A and B) or AI (results with Cs, Fig. 6C; similar results with OVA, data not shown) detected after aerosol challenge. These findings indicate that active allergic inflammation created by the last premating aerosol challenge is required for the maternal effect. In a second set of experiments, we administered neutralizing anti-IL-4 (24) or control rat IgG Ab to female mice just after completion of their last premating OVA aerosol (week 12; see Fig. 1). Offspring of anti-IL-4-treated mother mice showed markedly decreased AHR and AI compared with their IgG-treated counterparts, in sensitization and challenge protocols using either the same Ag (OVA; Fig. 7A) or a different Ag (Cs; Fig. 7, B and C).

These data implicate maternal IL-4 induced by active allergic inflammation in the period just before pregnancy as necessary for maternal transmission of susceptibility. However, we also considered the possibility that persistence and transfer of rat anti-IL-4 IgG from mother to offspring might result in functionally important levels of neutralizing Ab in the circulation of newborns. This could block development of AHR and AI in the offspring (similar to direct treatment with anti-IL-4 (29), but would preclude any interpretation of the role of IL-4 in earlier events in the mother.
mouse. To address this possibility, we first measured the concentration of rat IgG in serum samples taken from babies of treated mothers at day 3 after birth. As shown in Fig. 8A, we did detect circulating anti-IL-4 in these newborns (mean ± SE: 49 ± 8 ng/ml; n = 14). To test the functional effect, if any, of this persistent anti-IL-4, we measured the serum levels of baby mice on day 3 of life after injection with different amounts of rat anti-IL-4 on day 2. As shown in Fig. 8A, injection of 10 μg i.p. resulted in levels markedly higher (630 ± 67 ng/ml; n = 12) than that seen in serum of babies of mothers treated with 1 mg i.p. before mating. The functional effect of this amount of circulating anti-IL-4 was tested by i.p. injection on day 3 of 10 μg anti-IL-4 into a cohort of offspring of asthmatic mothers before i.p. injection with allergen on day 4, aerosol allergen challenge days 12–14, and analysis on day 15, as per the usual protocol. This treatment had no effect on the development of AHR in response to Cs (Fig. 8B), C, and eosinophilia (× 10^5) upon BAL (data not shown).

**Discussion**

Epidemiologic studies have identified an increased risk for asthma in children of asthmatic mothers, but the mechanism(s) for this effect has not been well characterized. This study sought to test the hypothesis that biologic transfer of mediator(s) from mother to child can cause increased susceptibility to development of allergic asthma. We used a mouse model of asthma, in which airway hyperresponsiveness and allergic pulmonary inflammation are seen after sensitization and aerosol challenge with the allergen OVA. By intentionally using a suboptimal sensitization protocol that fails to induce the asthma phenotype in normal offspring, we sought to determine whether a maternal background of OVA allergy would render baby mice more responsive to allergen. There were three major findings. First, the data did indeed show development of the asthma phenotype in offspring of asthmatic (but not normal) mother mice. The data provide the first direct demonstration that transfer of asthma susceptibility can occur by biologic mechanisms in a model wherein genetic and environmental influences are excluded. Second, this model demonstrated that the maternal effect is allergen independent. This finding argues against a critical role for transfer of Ag or specific Abs per se, although the data do not exclude the possibility that Ag-specific immunity can also be transferred under some conditions. Third, the studies of this model also demonstrated a requirement for active maternal allergic inflammation in the period just before or during early gestation, and a critical function for IL-4.

Some advantages and limitations of the experimental design merit discussion. Use of a mouse model allowed us to experimentally exclude two other potentially important mechanisms for maternal influence on asthma risk in humans: transmission of susceptibility genes and effects of maternal behavior (e.g., smoking). Consequently, the demonstration of maternal transmission of asthma risk in our model does not address the likely contribution of these other pathways to final outcomes in people. Mouse (and other animal) models of asthma are imperfect replicates of the human disorder. Nevertheless, analysis of the asthma phenotype in mice (airway hyperresponsiveness, allergic pulmonary inflammation, and allergen-specific Abs) has provided many useful insights into pathogenesis (27, 30–32).

Intervention with neutralizing Ab and manipulation of the model provided data that IL-4 induced by the last premating OVA challenge is a necessary mediator for maternal effects on offspring. We observed a remarkable persistence of the rat anti-mouse IL-4 in the serum of offspring of treated mother mice. These findings are similar to the long t_1/2 in mouse serum reported for rat anti-IL-5 (33). However, direct testing showed that 10-fold higher serum concentrations of anti-IL-4 were ineffective at blocking the susceptibility of newborn mice of allergic mothers to allergic sensitization, supporting an important mechanistic role for IL-4 at some point in the maternal/fetal interaction. Whether this represents persistence of IL-4 induced by the premating exacerbation of our protocol and subsequent transplacental transfer or an indirect effect of IL-4 on other immune cells and mediators that in turn
affect the developing individual remains to be determined. In addition, this model will be useful to further analyze the potential of other pharmacologic (e.g., anti-inflammatory) or public health (e.g., allergen avoidance) interventions on the maternal transmission of asthma risk.

Acknowledgments

We thank Drs. Stephanie Shore and Patricia Finn for their helpful advice.

References