

Duration of Allergic Susceptibility in Maternal Transmission of Asthma Risk

Alexey V. Fedulov, Adriana S. Leme, Lester Kobzik

Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA

Keywords

Duration, maternal asthma, OVA, sensitization

Correspondence

Alexey V. Fedulov, MD, PhD, Department of Environmental Health, Harvard School of Public Health, 665 Huntington Ave. HSPH-1, 1313. Boston, MA 02115, USA.
E-mail: afedulov@hsph.harvard.edu.

Submitted February 21, 2007;
accepted March 26, 2007.

Citation

Fedulov AV, Leme AS, Kobzik L. Duration of allergic susceptibility in maternal transmission of asthma risk. *Am J Reprod Immunol* 2007; 58:120–128

doi:10.1111/j.1600-0897.2007.00496.x

Problem

Asthma has its origins in early-life. Maternal, not paternal asthma is an important risk factor, but the mechanisms and the duration of the maternal effect are unknown.

Method of Study

Offspring of asthmatic and normal mice were sensitized with ovalbumin (OVA) at 1, 3, 6 and 10 weeks of age, challenged with aerosolized OVA 2 weeks later and tested for airway hyperresponsiveness (AHR) and allergic airway inflammation (AI).

Results

Offspring of asthmatic, but not normal, mothers showed AHR and AI after OVA sensitization at week 1. Similarly increased susceptibility to OVA was observed when sensitized at 3 or 6 weeks, although the magnitude diminished. Offspring sensitized at 10 and challenged at 12 weeks showed some AI but no AHR.

Conclusion

In offspring from asthmatic mothers increased allergic susceptibility persisted through the young adulthood, albeit with gradual decline, which suggests a long-lived but reversible skewing of the developing immune system.

Introduction

Asthma is a respiratory disorder with origins in early life.^{1–4} Pathogenic mechanisms include roles for genetic susceptibility as well as exposure to environmental factors.^{1,5,6} An additional factor, revealed by epidemiological studies, is that maternal asthma significantly increases the risk of developing the disease during childhood.^{7–10} This ‘maternal effect’ suggests that prenatal events also dramatically influence the early susceptibility to allergic airway disease.¹¹

We have previously described a mouse model of maternal transmission of asthma risk that allows focused investigations on immune mechanisms without the confounding genetic or environmental influ-

ences (e.g. smoking). Consistent with epidemiological data, babies of asthmatic BALB/c mother mice are more susceptible to develop asthma, even in response to an allergen (casein: CAS) unrelated to the ovalbumin (OVA) used to sensitize their mothers. This model thus excludes a critical role for transplacental passage of allergen-specific immunoglobulins produced by the mother.¹²

Potential pathophysiological mechanisms include perinatal transfer of maternal mediators such as cytokines, across placenta or via breast milk.¹³ Such mediators may polarize the newborn’s immune system, increasing its susceptibility to develop asthma by skewing immune reactions towards a pro-allergic Th2-driven response, to an even greater extent than is normally found.^{14,15} The duration of the ‘maternal

effect', i.e. the increased susceptibility in offspring of asthmatic mothers, is unknown.

Our prior experiments in this model analyzed development of the asthma phenotype in young mice (<3 weeks old).¹² However, we also observed some persistence of susceptibility (up to 6 weeks of age) in studies of CpG reversal of the maternal effect.¹⁶ This suggested that the mechanism behind maternal transmission of asthma risk may not be limited to a short-acting effect but rather can cause a pronounced and persistent skew in the newborn's immune system. Hence, we hypothesized that the suboptimal sensitization and challenge protocol may be still effective in offspring from asthmatic mothers even if sensitizing injection is given much later in life (Fig. 1). Therefore we investigated the duration of increased susceptibility to maternally transmitted asthma.

Material and methods

Animals

Newborn BALB/C mice were obtained commercially from Charles River Laboratories (Cambridge, MA, USA) as litters with their mother mouse at day 4 of age or by in-house breeding, as described below. Each mother mouse with its respective litter was housed separately, fed a commercially pelleted OVA-free 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-hr dark/light cycle. All experimentation was conducted under a protocol approved by our institutional review board.

Allergen Sensitization and Exposure

A detailed description of the original sensitization protocol can be found in ¹². Briefly, maternal sensitization was accomplished by i.p. injections of 0.1 mL

of PBS containing 5 µg of OVA and 1 mg of alum into mice at 5 and 9 days of age. After weaning, female mice were exposed to aerosols of OVA 3% (w/v) (ovalbumin grade V, Sigma-Aldrich, St Louis, MO, USA) in PBS, pH 7.4 for 10 min on three consecutive days at 4, 8, and 12 weeks of age (see Fig. 1). The aerosol exposure was performed within individual compartments of a mouse pie chamber (Braintree Scientific, Braintree, MA, USA) using a Pari IS2 nebulizer (Sun Medical Supply, Kansas City, KS, USA) connected to air compressor (Pulmo-AID; DeVilbiss, Somerset, PA, USA).¹⁶ Immediately after the last aerosol exposure, the female mice were placed in cages with normal males to allow mating. Babies from asthmatic mother mice (OVA sensitized twice and challenged before mating) received a single i.p. injection of OVA and alum on day 4 of life, an intentionally suboptimal sensitization as detailed in ¹². On days 13–15, they were exposed to aerosolized OVA, as above. This group is designated as 'AS2/As', indicating that mother was a 2-injection 'asthmatic' and the baby received 1-injection suboptimal protocol (see Table I). The suboptimal sensitization does not provoke any significant allergic response in babies born to normal mothers.^{12,16} Physiologic and pathologic analyses were performed on days 16 and 17, respectively. Control groups included: (i) babies born to normal mothers (not sensitized and challenged) subjected to the suboptimal sensitization (negative control, 'No/As'); and (ii) babies born to normal mothers, and sensitized by two injections of OVA and alum (days 4 and 8 of life), followed by aerosol challenge and evaluation 2 weeks later (positive control, 'No/AS2'). Unsensitized animals were always negative (As2/No), data not shown. The experimental protocol is summarized in Fig. 1. The coding of groups is summarized in Table I.

Similar protocols were used in order to investigate the duration of increased susceptibility. We chose three other timepoints for offspring suboptimal sensitization:

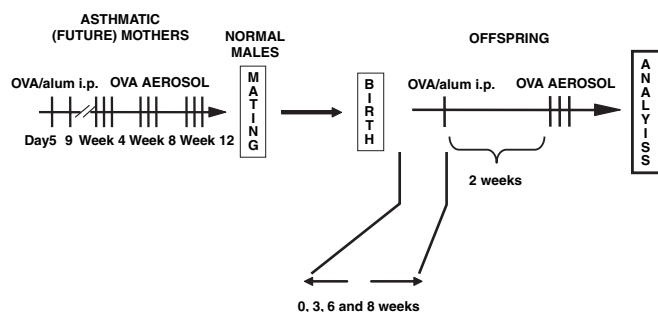


Fig. 1 Schematic summary of the experimental protocol. Babies from asthmatic mother mice [Ovalbumin (OVA)-sensitized twice and challenged before mating] received a single i.p. injection of OVA and alum at 0, 3, 6 or 10 weeks of age, followed by exposure to aerosolized OVA and analysis 2 weeks later.

TABLE I Group Coding

Group (Mother/offspring)	Definition	Role
No/As2	Normal mother; offspring received 2 injections of allergen and aerosol challenge	Positive control
No/As	Normal mother; offspring received 1 injection of allergen and aerosol challenge ('suboptimal' protocol)	Negative control
AS2/No	Asthmatic mother; normal unsensitized offspring, no aerosol challenge	Negative control
AS2/As	Asthmatic mother; offspring received 1 injection of allergen and challenge ('suboptimal' protocol)	Experimental groups

at 3, at 6 and at 10 weeks of life. These groups were challenged with aerosolized OVA 2 weeks later (at 5, 8, and 12 weeks of age, respectively; Fig. 1). Control groups were also studied: (i) babies born to normal mothers subjected to the suboptimal sensitization ('No/As'); and (ii) babies born to normal mothers, and sensitized by 2 injections of OVA ('No/AS2').

Pulmonary Function Testing

Twenty-four hours after the third day of exposure to OVA aerosol, the airway responsiveness of baby mice to increasing concentrations of aerosolized methacholine was measured using whole body plethysmography (Buxco, Sharon, CT, USA). Each mouse was placed in a chamber, and continuous measurements of box 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.^{17,18} After measurement of baseline Penh, aerosolized PBS followed by increasing concentrations of methacholine (6, 12, 25, 50, and 100 mg/mL) was nebulized through an inlet of the chamber for 1 min, and Penh values for 5 min after each nebulization were averaged and used to compare results across treatment groups.

Pathologic Analysis

After physiologic testing, the baby mice were killed with sodium pentobarbital (Veterinary Laboratories, Lenexa, KS, USA), and the trachea was cannulated. Bronchoalveolar lavage (BAL) was performed five times with 0.3 mL of sterile PBS instilled and harvested gently. Lavage fluid (recovery ~ 90% of instilled) was collected and centrifuged at 4°C (300 *g*) for 10 min, and the cell pellet was resus-

pended in 0.1 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, USA). These slides were fixed in 95% ethanol and stained with Diff-Quick (VWR, Boston, MA, USA), a modified Wright-Giemsa stain, and total of 100 cells were counted for each sample by microscopy. Macrophages, eosinophils, and lymphocytes were quantified.

After lavage, the chest wall was opened and the animals were exsanguinated by cardiac puncture. The lungs were removed and fixed in 10% buffered Formalin. After paraffin embedding, sections for microscopy were stained with H&E. An index of pathologic changes in coded H&E was derived by scoring the inflammatory cell infiltrates (eosinophils and mononuclear cells) around airways and vessels for greatest severity (0, normal; 1, <3 cell diameter thick; 2, 3–10 cells thick; 3, >10 cells thick) and overall extent (0, normal; 1, <10% of sample; 2, 10–25%; 3, >25%). The index was calculated by multiplying severity by prevalence, with a maximum possible score of 9.

Statistical Analysis

Data are expressed as mean $\pm$ S.E.M. One-way ANOVA with Newman-Keuls Multiple Comparison post test, or the Kruskal-Wallis multiple comparisons test with Dunn's post test were used as appropriate. For Penh data Repeated measures ANOVA was used: this method takes into consideration all of the repeated measures after consecutive doses of methacholine and returns the probability of error (*P*-value) for the whole comparison. Analysis was performed using GraphPad Prism version 3.0 for Windows (GraphPad Software, San Diego, CA, USA) and Statistica 6.5 for Windows (Statsoft, Tulsa, OK, USA). Statistical significance was accepted when $P \leq 0.05$.

Results

Maternal Transmission of Susceptibility to OVA-Induced Allergic Airways Disease

Consistent with our earlier results¹⁶ offspring from asthmatic mother mice (AS2/As) submitted to an intentionally suboptimal sensitization protocol (First

week for sensitization and week 2 for challenge, designated 0–2) showed increased susceptibility to OVA as seen by increased AHR in the methacholine test (Fig. 2a) and AI seen as eosinophila counts on BAL slides (Fig. 3a.) Histopathology showed accumulation of eosinophils and mononuclear cell infiltration around airways and vessels (Fig. 4a–d).

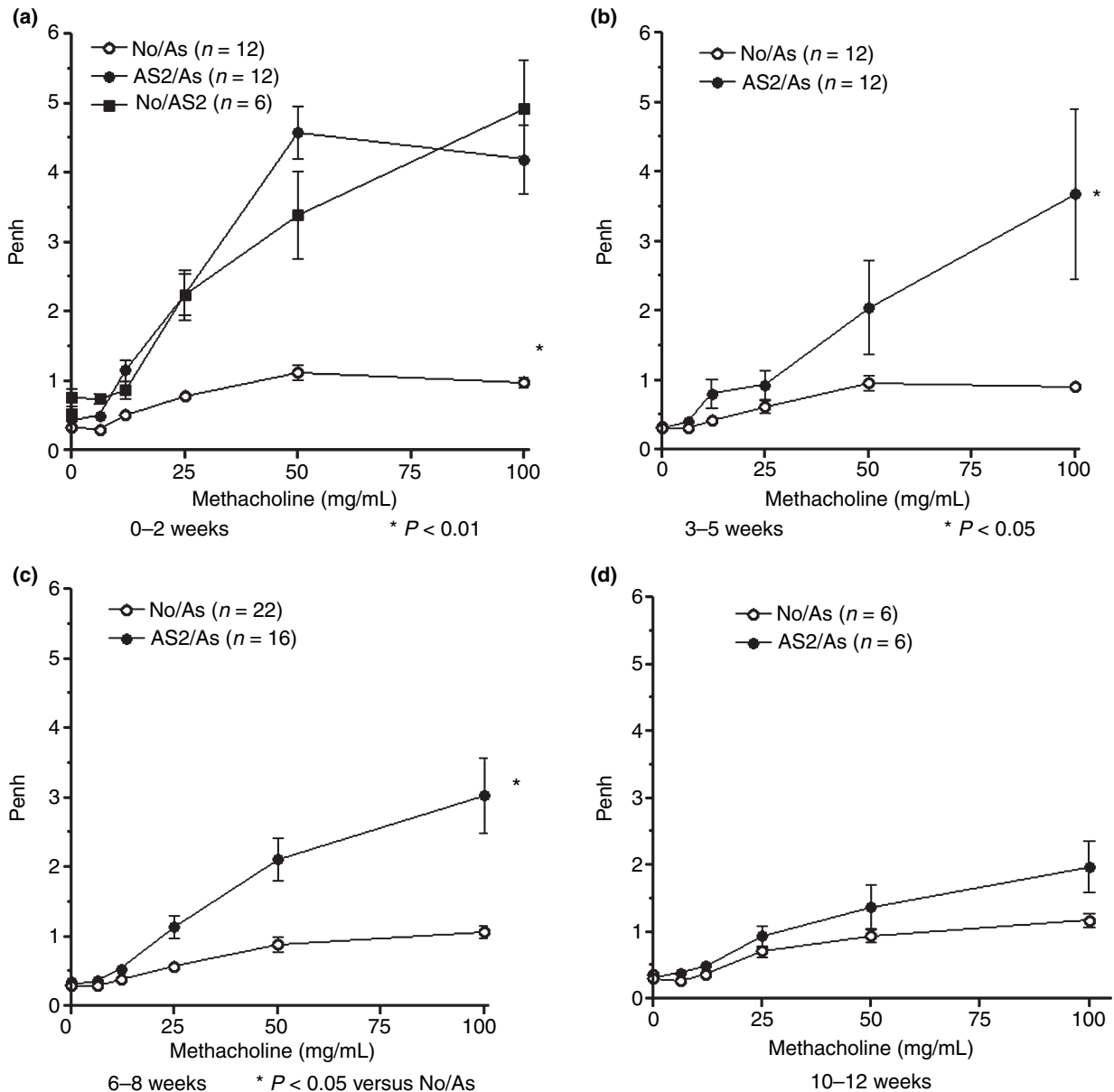


Fig. 2 Airway hyperresponsiveness (AHR). Babies from asthmatic mothers AS2/As (●, $n = 12$) showed increased AHR (Penh), $*P < 0.01$ versus No/As (○, $n = 12$) at 0–2 week timepoint (a); AHR was also increased with $*P < 0.05$ at 3–5 weeks (b) and at 6–8 weeks (c). At 10–12 week timepoint the difference was minor and not statistically significant (d). Changes in babies from normal mothers No/As (○, $n = 12$) remained minimal throughout. The positive control group included babies from normal mothers (No/AS2) which received two injections of OVA (positive control, ■, $n = 6$).

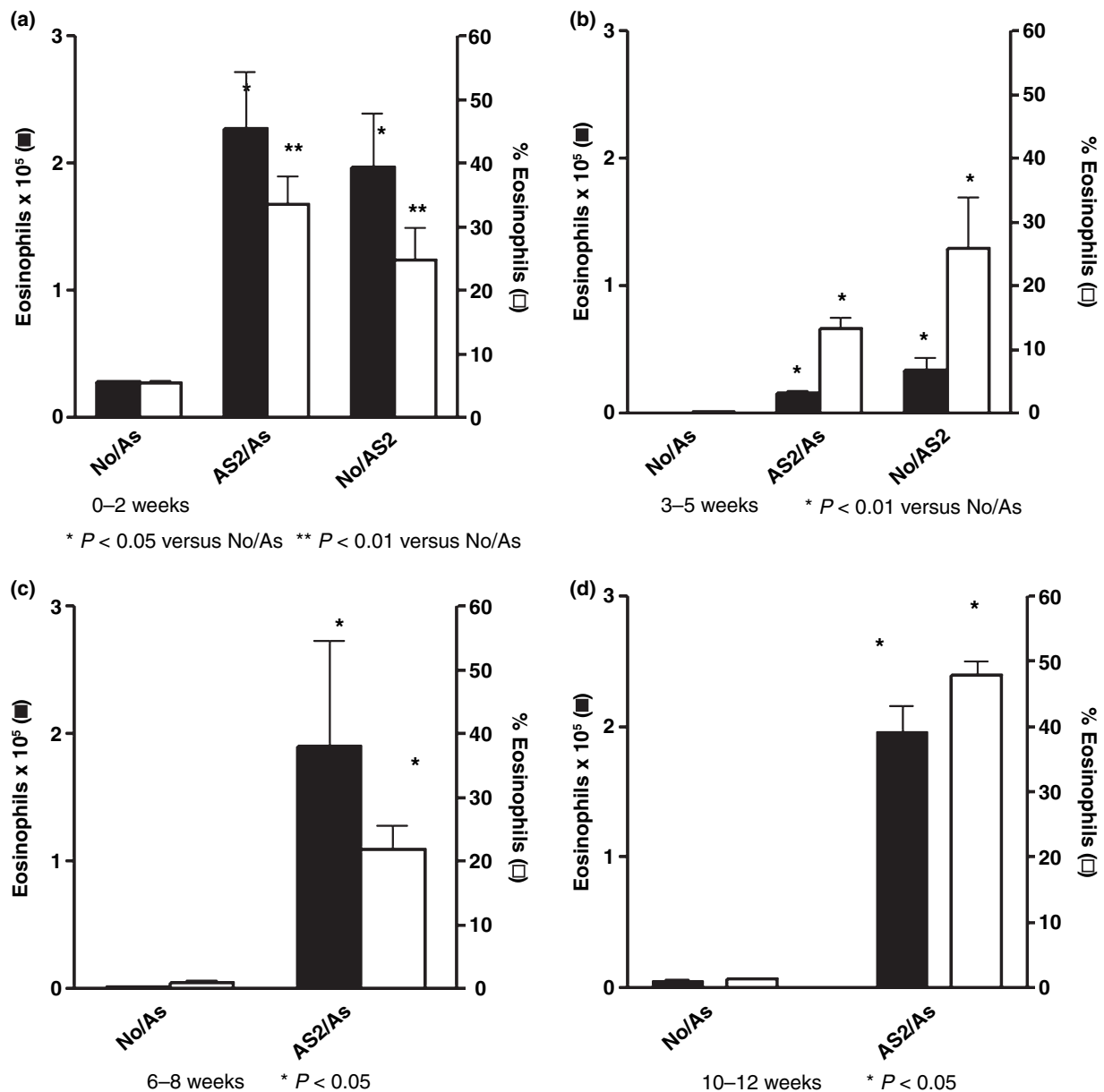
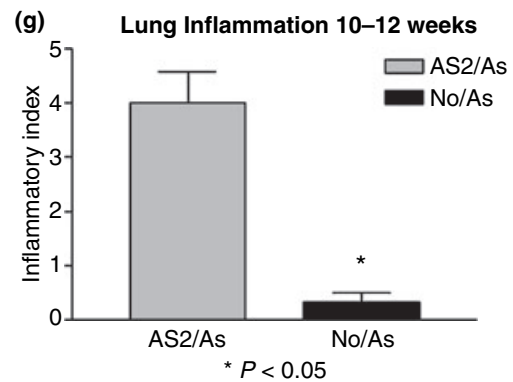
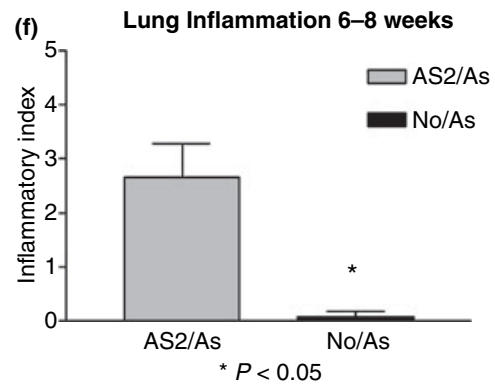
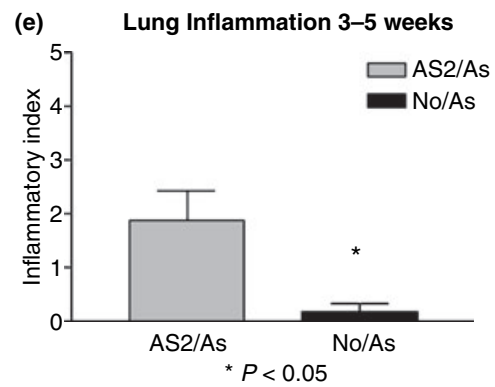
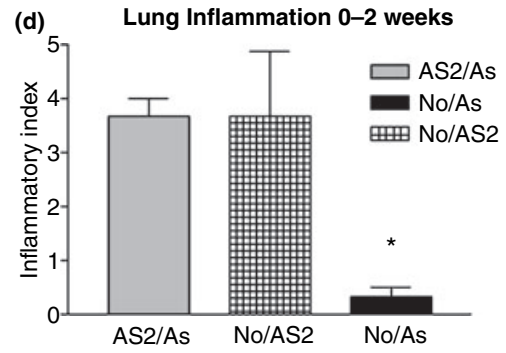
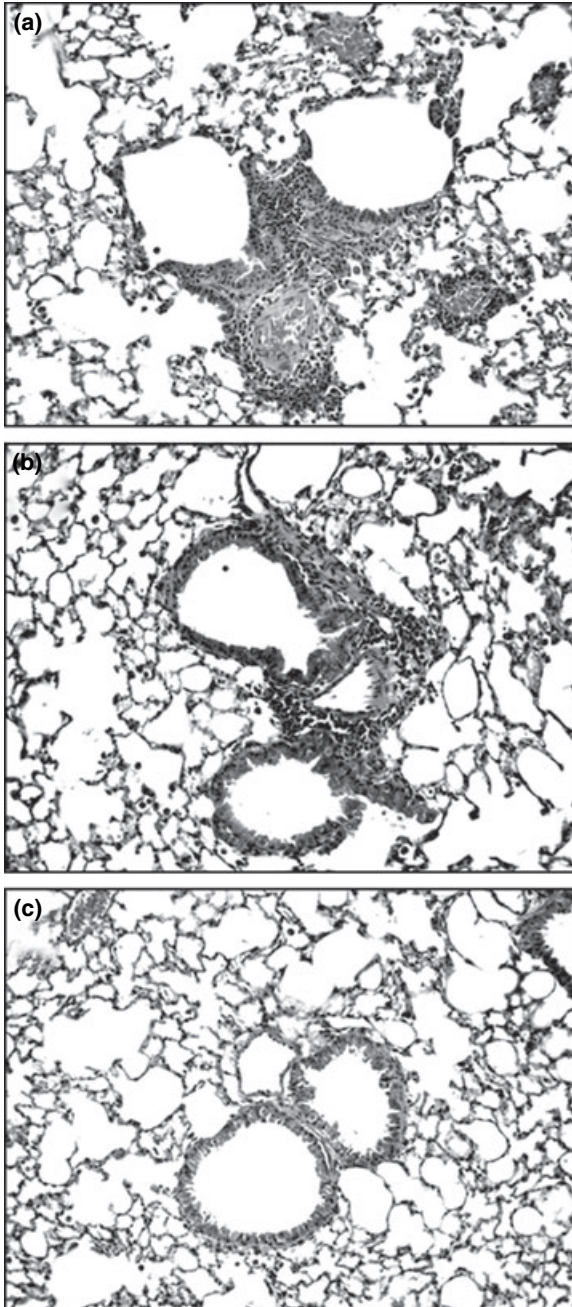


Fig. 3 Airway inflammation. Babies born to asthmatic mothers (group AS2/As), but not babies of normal mothers (No/As) had increased eosinophil counts in BAL samples at all timepoints.

Fig. 4 Lung histopathology. Histopathologic changes of allergic inflammation, such as accumulation of eosinophils and mononuclear cells around airways and vessels, were observed in (a) (offspring of asthmatic mothers, AS2/As), and (b) (positive controls, No/AS2), but not in (c) (offspring of normal mothers, No/As). (d–f): An index of pathologic changes in coded H&E lung slides was derived by scoring the inflammatory cell infiltrates around airways and vessels for greatest severity (0, normal; 1, <3 cell diameter thick; 2, 3–10 cells thick; 3, >10 cells thick) and overall extent (0, normal; 1, $<10\%$ of sample; 2, 10–25%; 3, $>25\%$). The index was calculated by multiplying severity by prevalence, with a maximum possible score of 9. * $P < 0.05$ versus No/As.



Two groups represent positive and negative controls for the investigation of maternal effects. The positive control group, comprising babies born to normal mother mice and sensitized by two injections of OVA (No/AS2), reproducibly showed: (i) AHR to methacholine (increased Penh, Fig. 2); (ii) increased eosinophils on BAL (Fig. 3); and (iii) intense histopathologic changes (Fig. 4), such as accumulation of eosinophils and mononuclear cell infiltration around airways and vessels. Results of semiquantitative scoring of histopathologic changes corroborate the qualitative changes and are summarized in Fig. 4. These results contrast to those seen in the negative controls: babies from normal mother mice subjected to the suboptimal sensitization (No/As) show neither AHR nor AI (Figs 2–4).

Duration of Allergic Susceptibility to Asthma

Offspring of asthmatic mothers and control mice were subjected to a similar protocol, but the time of initial allergen exposure after birth was varied. Specifically, babies from asthmatic (AS2/As) and normal mothers (No/As) were sensitized with OVA at 3, 6 and 10 weeks of age, followed by challenge with aerosolized OVA and analysis 2 weeks later, designated further as 3–5 weeks, 6–8 weeks, and 10–12 weeks.

Increased AHR (Fig. 2), increased BAL eosinophilia (Fig. 3), and pulmonary inflammation (Fig. 4) were observed in offspring first sensitized at 3 weeks (group 3–5) as well as 6 weeks of age (group 6–8). These outcomes were seen only in the offspring of asthmatic, but not normal mothers. Positive controls with two injections of OVA and alum (No/AS2) remained highly positive throughout the study (data not shown). Offspring of asthmatic mice sensitized at 10 weeks of age and allergen-challenged at 12 weeks did not develop significant AHR, but the AI persisted (Figs 2d and 3d). Although the changes in the 3–5 and 6–8 weeks groups were qualitatively similar to the results seen in the 0–2 ‘original protocol’ babies, we observed a trend for decreasing intensity of AHR (Fig 2) and inflammation (Figs 3 and 4), as illustrated by the comparison of AHR data across the groups shown in Fig. 5.

Discussion

We investigated the duration of increased allergic susceptibility in a model of maternal transmission of asthma risk. We used the mouse model of maternal

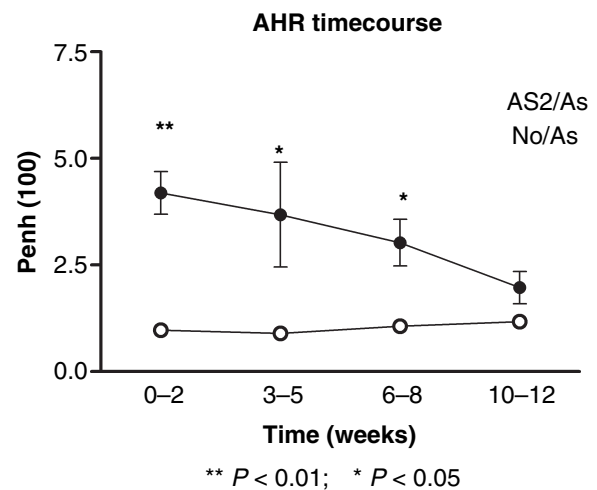


Fig. 5 Time course of airway hyperresponsiveness (AHR). The magnitude of AHR seen in offspring of asthmatic mother mice gradually decreases over time (age of sensitization), as shown in the Penh response to 100 mg/mL of methacholine. ** $P < 0.01$, * $P < 0.05$.

transmission previously developed in our laboratory, in which offspring from ‘asthmatic’ mother mice sensitized and exposed repeatedly to aerosolized OVA show increased susceptibility to this and other allergens.¹²

The key finding is that offspring of asthmatic (but not normal) mice develop a full asthma phenotype (AHR and allergic inflammation) even if they are initially sensitized as late as 6 weeks of age. Moreover, some changes in the immune response are still evident when sensitization occurs at 10 weeks after birth, although there is evidence of a gradual decline in the magnitude of the maternal effect. This includes the lack of AHR in the mice first sensitized at 10 weeks of age, and the relative decline of the absolute number of eosinophils in lavage samples despite increases in overall body weight as the animals’ age. The data suggest that the changes to the neonate’s immune system caused by as yet unidentified maternal factors do not require their sustained presence, since the effect lasts for more than 6 weeks, well past the breast-feeding and weaning age into murine adulthood.

Although it has been noted that age at first allergen exposure is an important factor in response,¹⁹ no prior experiments have specifically addressed how long maternally transmitted susceptibility may persist in the organism. However, it has been recently found by Gelfand et al.²⁰ that sensitization of ‘young’ mice (1–20 weeks of age), but not older animals

(30–40 weeks), led to the development of AHR, airway eosinophilia, Th2 cytokine responses, and allergen-specific IgE, independent of the age when the challenge was conducted, thus showing that the age at the time of initial sensitization was a critical factor determining the response to later allergen challenge. Additional data also provide a context for the findings reported here and support the idea that immune system changes *in utero* or in early post-natal life can cause persistent or significant alterations in allergic susceptibility. These data include ideas of systemic maternal-mediated skewing of fetal and early neonatal immunity toward Th2 responses,²¹ such as: the requirement for a successful pregnancy of increased Th2/Th1 cytokines ratio,²² the demonstrated synthesis of Th2 cytokines at the placental interface,²³ and the observation of increased production of Th2 cytokines by fetal or neonatal T cells.^{14,24} The potential of post-natal influences from breast milk to contribute to the maternal effect has been reported earlier by our laboratory.¹³

Our findings are consistent with the epidemiologic observations that asthma, even in adults, is most often initiated in early childhood.^{25,26} The data presented here demonstrate experimentally that *in utero*/early-life maternal influences can result in increased allergic susceptibility leading to the onset of asthma in adulthood, even when these influences are not actively present. It is important to note that precise extrapolation to human biology from mouse models is difficult. For example, the rapid postnatal development and weaning to a reproduction-competent adult mouse in 6 weeks with a life span of ~2 years is clearly different from the human experience. Nevertheless, animal models can inform understanding of mechanisms of human disease. The data presented here indicate that the asthma susceptibility previously studied mainly in young mice is a robust and persistent effect, and not a transient one, and will allow additional experimentation to test interventional therapies and to explore mechanisms.

Acknowledgments

This work was supported by National Institutes of Health Grant HL69760.

References

- Gern JE, Lemanske RF, Busse WW: Early life origins in asthma. *J Clin Invest* 1999; 104:837–843.
- Holt PG, Jones CA: The development of the immune system during pregnancy and early life. *Allergy* 2000; 55:688–697.
- Wright AL: The epidemiology of the atopic child: who is at risk for what? *J Allergy Clin Immunol* 2004; 113:S2–S7.
- Vance GHS, Holloway JA: Early life exposure to dietary and inhalant allergens. *Pediatr Allergy Immunol* 2002; 13(Suppl. 15):14–18.
- Holloway JW, Beghe B, Holgate ST: The genetic basis of atopic asthma. *Clin Exp Allergy* 1999; 29:1023–1032.
- von Mutius E, Eder W: Genetics in asthma: the solution to a lasting conundrum? *Allergy* 2005; 60:1482–1484.
- Ruiz RG, Kemeny DM, Price JF: Higher risk of infantile atopic dermatitis from maternal atopy than from paternal atopy. *Clin Exp Allergy* 1992; 22:762–766.
- Litonjua A, Carey V, Burge H, Weiss S, Gold D: Parental history and the risk of childhood asthma: does mother confer more risk than father? *Am J Respir Crit Care Med* 1998; 158:176–181.
- Illi S, von Mutius E, Lau S, Nickel R, Niggemann B, Sommerfeld C, Wahn U: The pattern of atopic sensitization is associated with the development of asthma in childhood. *J Allergy Clin Immunol* 2001; 108:709–714.
- Liu CA, Wang CL, Chuang H, Ou CY, Hsu TY, Yang KD: Prenatal prediction of infant atopy by maternal but not paternal total IgE levels. *J Allergy Clin Immunol* 2003; 112:899–904.
- Prescott SL: Maternal allergen exposure as a risk factor for childhood asthma. *Curr Allergy Asthma Rep* 2006; 6:75–80.
- Hamada K, Suzaki Y, Goldman A, Ning YY, Goldsmith C, Palecanda A, Coull B, Hubeau C, Kobzik L: Allergen-independent maternal transmission of asthma susceptibility. *J Immunol* 2003; 170:1683–1689.
- Leme AS, Hubeau C, Xiang Y, Goldman A, Hamada K, Suzaki Y, Kobzik L: Role of breast milk in a mouse model of maternal transmission of asthma susceptibility. *J Immunol* 2006; 176:762–769.
- Prescott S, Macaubas C, Holt B, Smallacombe T, Loh R, Sly P, Holt P: Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998; 160:4730–4737.
- Szefalusi Z, Nentwich I, Gerstmayr M, Jost E, Todoran L, Gratzl R, Herkner K, Urbanek R: Prenatal allergen contact with milk proteins. *Clin Exp Allergy* 1997; 27:28–35.
- Fedulov A, Silverman E, Xiang Y, Leme A, Kobzik L: Immunostimulatory CpG oligonucleotides abrogate

- allergic susceptibility in a murine model of maternal asthma transmission. *J Immunol* 2005; 175:4292–4300.
- 17 Lutchen KR, Yang K, Kaczka DW, Suki B: Optimal ventilation waveforms for estimating low-frequency respiratory impedance. *J Appl Physiol* 1993; 75:478–488.
 - 18 Hantos Z, Daroczy B, Suki B, Nagy S, Fredberg JJ: Input impedance and peripheral inhomogeneity of dog lungs. *J Appl Physiol* 1992; 72:168–178.
 - 19 Kips JC, Anderson GP, Fredberg JJ et al. Murine models of asthma. *Eur Respir J* 2003; 22:374–382.
 - 20 Gelfand EW, Joetham A, Cui ZH, Balhorn A, Takeda K, Taube C, Dakhama A: Induction and maintenance of airway responsiveness to allergen challenge are determined at the age of initial sensitization. *J Immunol* 2004; 173:1298–1306.
 - 21 Busse WW, Rosenwasser LJ: Mechanisms of asthma. *J Allergy Clin Immunol* 2003; 111:S799–S804.
 - 22 Wegmann TG, Lin H, Guilbert L, Mossmann TR: Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol Today* 1993; 14:353–356.
 - 23 Lin H, Mossmann TR, Guilbert L, Tuntipopipant S, Wegmann TG: Synthesis of T helper 2-type cytokines at the maternal-fetal interface. *J Immunol* 1993; 151:4562–4573.
 - 24 Warner JA, Jones AC, Miles EA, Warner JO: Prenatal sensitization. *Pediatr Allergy Immunol* 1996; 7:98–101.
 - 25 von Mutius E: The burden of childhood asthma. *Arch Dis Child* 2000; 82:II2–5.
 - 26 Weiss ST: Epidemiology and heterogeneity of asthma. *Ann Allergy Asthma Immunol* 2001; 87:5–8.