

Characteristic Features of Allergic Airway Inflammation in a Murine Model of Infantile Asthma

Yasushi Ohki Kenichi Tokuyama Hiroo Mayuzumi Akira Sato Harumi Koyama
Takumi Takizawa Hirokazu Arakawa Hiroyuki Mochizuki Akihiro Morikawa

Department of Pediatrics and Developmental Medicine, Gunma University Graduate School of Medicine, Maebashi, Japan

Key Words

Cytokines · Allergic airway inflammation · Maturation

Abstract

Background: The pathophysiology of infantile asthma may differ from that in older children or in adults, partly because of the different immune response depending upon maturation. In adult mice, the sensitizing dose of antigen is known to be critical to the polarized development of helper T cell subsets and allergic airway inflammation. We wanted to know the characteristics of allergic airway inflammation of infantile asthma by developing a murine model. **Methods:** BALB/C mice at different stages of maturation (juvenile: 3 days after birth; adult: 8 weeks of age) were sensitized with 10 or 1,000 µg ovalbumin (OVA) or vehicle. The animals were then challenged with aerosolized OVA or saline once a day during 6 consecutive days. After the final challenge, bronchial hyperresponsiveness (BHR), bronchoalveolar lavage fluid (BALF), histological changes in the airways and immunological status were examined. **Results:** In both juvenile and adult animals, sensitization with 10 µg OVA induced the T helper 2 response (elevated IL-4 and decreased IFN-γ levels). BHR, airway eosinophilia, the inflammatory cell infiltration, goblet cell metaplasia (GCM), and IgE antibody production were more prominent in animals given this dose than 1,000 µg OVA. Among

these responses, GCM as well as BALF IL-4, and BHR were comparable between juvenile and adult animals, whereas other parameters were lower in juvenile animals, especially in those given 1,000 µg OVA. **Conclusion:** GCM and, consequently, airway mucus hypersecretion may be an important component of allergic airway inflammation in infantile asthma.

Copyright © 2005 S. Karger AG, Basel

Introduction

Recently, asthma is believed to be a chronic inflammatory airway disease, mainly based on findings of adult patients [1]. However, longitudinal studies indicate that about 50% of all asthmatic children are virtually free from symptoms from 10 to 20 years [2], whereas this is the case in only about 10% of asthmatic adults [3]. These studies support the hypothesis that the pathophysiology of asthma may differ depending on age. In the Global Initiative for Asthma (GINA) 2002 [1], it is recommended that the diagnosis and the management of asthma in children must be considered in their own right, and not merely extrapolated from experience with adults.

In asthma during childhood, asthma in infants (so-called infantile asthma) deserves unique clinical remarks. First, acute asthma in infants tends to exacerbate rapidly, partly because of anatomical and physiological character-

istics. Second, bronchodilators are less effective against acute exacerbations in infants compared to older children [4]. Thus, the symptoms of infantile asthma are often difficult to control. In view of this point, it is important to know the specificities of the pathophysiology of infantile asthma for its proper management. Lung function testing and examination of sputum contents may be helpful for analyzing the pathophysiology. However, this is unlikely, because it is difficult to get cooperation from infants. Thus, an animal model may be a useful tool to understand infantile asthma.

Most patients with infantile asthma are known to be atopic. The pathophysiology of atopic asthma is characterized by airway eosinophilia, an elevated serum IgE level, and bronchial hyperresponsiveness (BHR). In addition, so-called T helper 2 (Th2) cytokines, such as interleukin (IL)-4, 5, and 13 produced by this helper T cell subset, are thought to play a pivotal role in this process [5, 6]. The Th2 deviation has been reported to be induced by several factors, including the genetic background, the doses of antigen for sensitization, and the cytokines present during early T cell activation [6]. The dose of antigen is assumed to be one of the important factors in Th2 cell development and the development of airway allergic inflammation in the adult mouse [7].

Clinically, each infant may be sensitized by different amounts of antigens. This may partly determine the direction of the development of allergic airway inflammation in each patient. Thus, we wanted to know whether characteristic features of allergic airway inflammation in juvenile animals, a model of infantile asthma, might differ from those in adult animals under a different sensitization process.

Materials and Methods

Animals

BALB/C mice at different stages of maturation (juvenile mouse: 3 days after birth, and adult mouse: 8 weeks of age) were utilized in this study. Eight-week-old adult mice and pregnant mice were obtained from Charles River Japan (Shizuoka, Japan). After delivery of newborn mice, each mother and her litter were housed separately. All experimental animals used in this study were treated according to a protocol approved by the institutional animal care and use committee.

Sensitization and Airway Challenge Protocol

Adult and juvenile mice were randomly divided into OVA group and control group. Mice were immunized intraperitoneally with 10 or 1,000 µg OVA (grade V, Sigma, USA) in 20 mg of alum (Al(OH)₃) on day 0 and boosted on days 7 and 14 as described previously [8]. Thereafter, they were challenged with aerosolized 2.5%

OVA solution using an ultrasonic nebulizer (NE-U12, Omron, Tokyo, Japan) in a 4.5-liter inhalation box for 30 min a day on 6 consecutive days (from day 15 to day 20). Nonimmunized control mice received 3 injections of alum alone and were repeatedly challenged with vehicle (saline) under the same schedule. Therefore, 6 experimental groups: [0 (control), 10 and 1,000 µg OVA-sensitized juvenile or adult mice] were constituted (n = 8–10 for each group). The measurement of BHR, the number of cells in BALF, and histological studies were conducted 48 h after the final inhalation challenge.

Determination of BHR

BHR to increasing concentrations of aerosolized methacholine (Mch) was studied on unstrained conscious mice as described previously [9]. The mice were placed in a barometric plethysmographic chamber (Buxco electronics, Sharon, Conn., USA), and the pressure-time wave was continuously measured. The main indicator of airflow obstruction, enhanced pause (P_{enh}), which shows a strong correlation with airway resistance, was calculated. The mice were challenged with Mch (3.13, 6.25, 12.50, and 25.00 mg/ml) aerosol generated by an ultrasonic nebulizer (NE-U12, Omron, Tokyo, Japan). Respiratory mechanics were measured for 3 min after each aerosol inhalation and averaged. BHR was evaluated utilizing 3 parameters, (1) Mch concentration required for 100% P_{enh} increase from the baseline value referred to as PC200, (2) the leftward shift of the dose-response curve, (3) the absolute value of P_{enh} corresponding to the maximum Mch concentration (25.00 mg/ml) referred to as maximum reactivity (MaxR).

The Sampling Procedure of Blood and BALF

After assessment of BHR, the animals were killed with an overdose of pentobarbital (50 mg/animal i.p.) to obtain serum samples. After getting the samples, a 24-gauge cannula was introduced into the proximal portion of the trachea, and the lungs were lavaged 3 times with phosphate-buffered saline (PBS, 0.4 ml for adult and 0.3 ml for juvenile mice). The BALFs were centrifuged at 800 rpm for 5 min. The supernatant was preserved for measurement of cytokine levels at -70°C . The cell pellet was resuspended in 0.3 ml of RPMI-1640 medium (Sigma). The total cell counts were performed with a hemocytometer, and the differential cell counts were performed on cytopspin preparations stained with Diff-Quick (Kokusai-Siyaku, Tokyo, Japan). A blinded observer counted a minimum of 200 cells for each sample.

Tissue Preparation

After getting BALFs, the lungs were inflated at 25 cm H₂O pressure. The trachea was clamped until fixation was completed. Tissue specimens were cut into 6-µm-thick sections of a mid-sagittal slice and embedded in paraffin and stained with hematoxylin and eosin (HE) and Alcian blue/periodic acid-Schiff (AB/PAS). The slides were coded and graded in a blind fashion, and the degrees of the inflammatory cell infiltration and goblet cell metaplasia (GCM) were examined.

Evaluation of the Inflammatory Cell Infiltration

The inflammatory cell infiltration into the lung was evaluated with a modification of a reproducible scoring system described previously [10]. A value from 0 to 3 per criterion was adjudged to each tissue section scored. Three criteria were scored to document the pulmonary inflammation: peribronchial inflammation, perivascu-

lar inflammation, and alveolar inflammation. For peribronchial and perivascular lesions, a value of 0 was adjudged when no inflammation was detectable, a value of 1 for occasional cuffing with inflammatory cells, a value of 2 when most bronchi or vessels were surrounded by a thin layer (1–5 cells thick) of inflammatory cells and a value of 3 when most bronchi or vessels were surrounded by a thick layer (>5 cells) of inflammatory cells. For assessing alveolar wall inflammation, a value of 1 was adjudged for increased numbers of inflammatory cells in alveolar walls, a value of 2 for 1–3 foci per section showing cellular alveolar exudate and atelectasis, and a value of 3 when more than 3 foci per section showing cellular alveolar exudate and atelectasis were observed additionally. The total score (the cellular infiltration score) was evaluated as the sum of these three subscores. Therefore, it ranged from 0 to 9.

Evaluation of GCM

The degree of GCM was evaluated by 2 different methods using either a semi-quantitative technique, in which the degree of GCM was analyzed, or a quantitative one, in which the proportion of mucus areas in the epithelial layer was evaluated.

(1) A semi-quantitative 5-point scale (mucus cell score): the slides stained by AB/PAS were examined with a light microscope (IX 70, Olympus Co., Tokyo, Japan). A semi-quantitative 5-point scale was performed by a previously described method [11], i.e. grade 0 = 0%, grade 1 = 0–25%, grade 2 = 25–50%, grade 3 = 50–75%, and grade 4 = 75–100% of epithelial cells positively stained by AB/PAS, respectively. The mean of the grade in the main bronchus and the large membranous airways was scored separately in each animal. The average of both points was referred to as the mucus cell score.

(2) A quantitative measure of the mucous substance area in the epithelium (mucus area): The percent mucosubstance area in the epithelium was determined as described previously [12]. In brief, images of the membranous airways were recorded through a light microscope (IX 70, Olympus Co.) that was connected to a color chilled CCD camera system (M 3204C, Olympus). Ten random points on the area of AB/PAS-stained mucosubstance within the epithelium were selected. Thereafter the color data of selected pixels were divided into 3 color components (hue, saturation, and intensity). The total area with same color threshold was measured automatically by the graphic analysis software (Win ROOF vers. 3.41, Mitani Co., Fukui, Japan). The data were expressed as the mucus area, which was calculated according to the following formula:

$$\text{Mucus area (\%)} = (\text{mucus area/total epithelial area}) \times 100$$

Determination of OVA-Specific Antibodies

OVA-specific IgE and IgG2a levels were determined by ELISA as described previously [8]. Ninety-six-well microtiter plates were coated with 200 µg/ml of OVA (grade V, Sigma, USA) diluted in 0.1 M NaHCO₃. After 2 h of incubation at 37°C, plates were washed with washing buffer (Sigma) and blocked with PBS-BSA for 2 h at 37°C. After washing with the buffer 5 times, serially diluted 100-µl serum samples were added and incubated for 2 h at 37°C. Plates were washed 5 times with 300 µl of the washing buffer. Then 100 µl of 1:800 diluted rat anti-mouse IgE monoclonal antibody (Biosource International, Calif., USA) or rat anti-mouse IgG2a monoclonal antibody (Biosource International) were added. After 2 h of incubation at 37°C, plates were washed 5 times with 300 µl of the washing buffer. After 2 h of incubation at 37°C, the reaction

chromogen was generated with FAST (Sigma). After the reaction was stopped with H₂SO₄, plates were read in a multiplate reader at 490 and 620 nm. The serum pooled from adult mice that were sensitized and challenged with OVA was used as a positive control. The OVA-specific IgE titer was determined and expressed as the reciprocal of the highest dilution giving a positive value. The OVA-specific IgG2a antibody titer was normalized relative to the absorbance of positive control serum and expressed as percent of positive control serum.

Measurement of BALF Cytokines

In order to examine Th1/Th2 balance in the airways of 6 groups studied, BALF were collected 24 h after the final challenge in selected animals of each group (n = 4–6), and IL-4 and IFN-γ levels were measured by commercially available ELISA kits (Endogen, Boston, Mass., USA). The detection limit was 15 pg/ml for both cytokines. We have selected this time point to evaluate BALF cytokine levels according to the report by Ohkawara et al [13], in which the time course of their levels were examined.

Statistical analysis

All data were expressed as mean ± SEM unless otherwise specified. A non-parametric analysis of variance (Kruskal-Wallis test for unmatched pairs) was used to determine the significance of the variance between groups. If a variance was found to be significant, a Mann-Whitney U-test was performed to assess the significance of differences between groups. A p value of less than 0.05 was considered to indicate statistical significance. In the case of multiple comparisons, a Bonferroni correction was applied. The statistical analysis was performed utilizing Statview version 4.5 (Abacus Concepts, Inc., Berkeley, Calif., USA).

Results

Six experimental groups were constituted (n = 8–10 mice for each group), and no mouse died during the study period.

BALF Cytokine Levels

In both juvenile and adult mice, a significant increase in IL-4 level was found in animals given 10 µg OVA. In animals given 1,000 µg OVA, only a slight but significant increase was seen in juvenile animals, although no increase was observed in adult animals (table 1). IFN-γ level decreased in both juvenile and adult animals given 10 µg OVA, with a significant difference in the latter compared to control animals. In animals given 1,000 µg OVA, no difference was found compared to control animals of the corresponding ages.

Analysis of OVA-Specific Antibodies

In adult animals, OVA-specific IgE level was significantly elevated in both groups given 10 and 1,000 µg OVA for sensitization, with a significantly greater degree

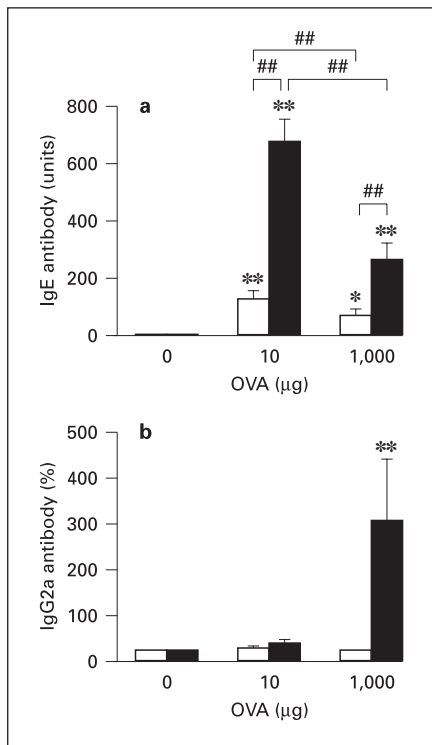


Fig. 1. OVA-specific IgE (a) and IgG2a levels (b). The open and closed columns indicate the values of juvenile and adult animals, respectively. Values are expressed as mean $\pm$ SEM. * $p < 0.05$ and ** $p < 0.01$ vs. control animals of the corresponding ages, respectively. ## $p < 0.01$ between groups ($n = 8-10$ mice for each group).

in the former than the latter group (fig. 1a). This was also the case in juvenile animals. Significantly higher IgE levels were observed in adult mice than in juvenile ones given corresponding doses of the antigen (fig. 1a). OVA-specific IgG2a levels were significantly elevated only in adult mice given 1,000 μg OVA compared to control animals given vehicle for OVA (fig. 1b).

Bronchial Responsiveness to Mch

Figure 2 shows the results of bronchial responsiveness to Mch in adult and juvenile mice. In adult animals, a marked leftward shift of the dose-response curve, a decrease in PC200, and an increase in MaxR were observed in those given 10 μg OVA. The degree of these changes in adult animals given 1,000 μg was milder than in those given 10 μg OVA (fig. 2b). In juvenile mice, a similar re-

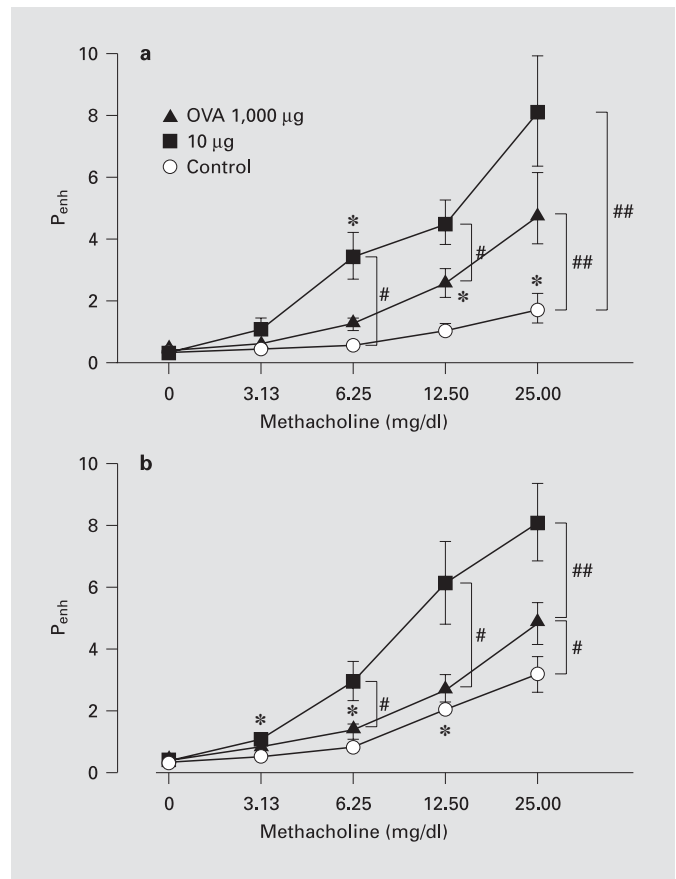


Fig. 2. Bronchial responsiveness to aerosolized methacholine in juvenile (a) and adult mice (b). Dose-response curves in animals given 10 and 1,000 μg OVA and vehicle (control) are shown. Values are expressed as mean $\pm$ SEM. # $p < 0.05$ and ## $p < 0.01$ between groups. * Indicates PC200 for each group of animals ($n = 8-10$ mice for each group).

Table 1. IL-4 and IFN- γ levels in BALF

Mice	OVA dose, μg		
	0	10	1,000
IL-4, ng/ml BALF			
Juvenile	0.0 $\pm$ 0.0	121.6 $\pm$ 62.1*	18.8 $\pm$ 6.8*
Adult	8.6 $\pm$ 5.6	88.5 $\pm$ 38.8*	14.6 $\pm$ 8.3
IFN- γ , ng/ml BALF			
Juvenile	75.5 $\pm$ 32.5	50.8 $\pm$ 33.1	86.2 $\pm$ 26.2
Adult	120.6 $\pm$ 29.8	13.5 $\pm$ 13.5*	77.7 $\pm$ 36.0

Values are expressed as mean $\pm$ SEM.

* $p < 0.05$ vs. the corresponding animals given vehicle for OVA (control) ($n = 4-6$ mice for each group.)

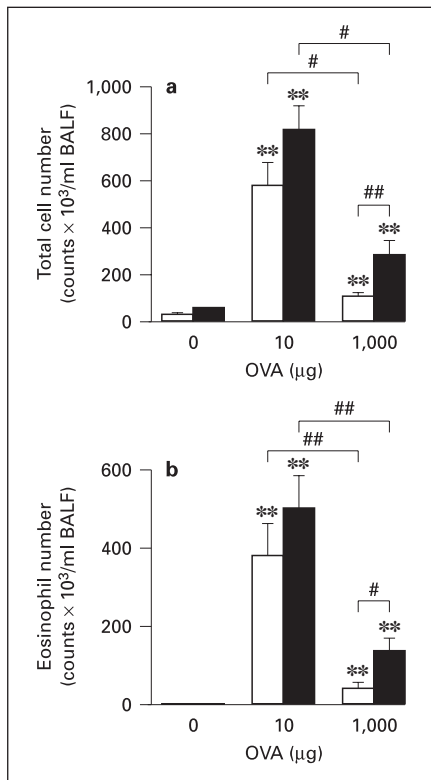


Fig. 3. The numbers of total cells (**a**) and eosinophils (**b**) in BALF. The open and closed columns indicate the values of juvenile and adult animals, respectively. Values are expressed as mean $\pm$ SEM. ** $p < 0.01$ vs. control animals with the corresponding age. # $p < 0.05$ and ## $p < 0.01$ between groups (n = 8–10 mice for each group).

lation was observed between animals given 10 μ g and 1,000 μ g OVA (fig. 2a). The degree of the decrease in the PC200 value showed a similar tendency in juvenile and adult animals; that is, in both groups of animals, the values of PC200 in animals given 10 and 1,000 μ g OVA were 4-fold and 2-fold less than the value in control animals, respectively.

Number of Inflammatory Cells in BALF

In both adult and juvenile mice, the number of total cells and eosinophils was markedly higher in animals given 10 μ g than in those given 1,000 μ g OVA (fig. 3a, b, respectively). In juvenile animals given 1,000 μ g OVA, the increases in the number of total cells and eosinophils were scarce compared with the other 3 groups of animals given OVA.

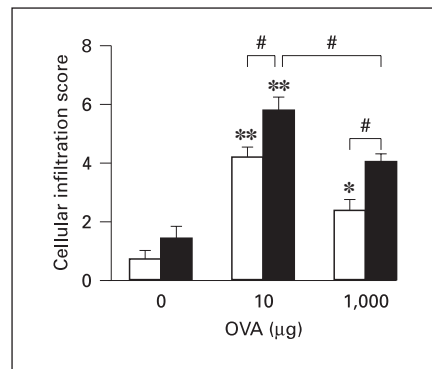


Fig. 4. Cellular infiltration score. The open and closed columns indicate the values of juvenile and adult animals, respectively. Values are expressed as mean $\pm$ SEM. * $p < 0.05$ and ** $p < 0.01$ vs. control animals with corresponding ages, respectively. # $p < 0.05$ between groups (n = 8–10 mice for each group).

Histopathological Findings of the Lung

In both adult and juvenile mice, the cellular infiltration score was more prominent in animals given 10 μ g OVA (fig. 4). The degree was significantly greater in adult mice than in juvenile ones given corresponding doses of OVA.

GCM was found in all groups of animals sensitized and challenged with OVA. Representative photographs are shown in figure 5. The result of the mucus cell score is shown in table 2. The degree of the increase was more prominent in animals given 10 μ g than in those given 1,000 μ g OVA in both ages, although there was no statistical difference between them. In contrast to the cellular infiltration score, the mucus cell score in juvenile mice was similar to that in adults given the corresponding doses of OVA (table 2). The mucus cell score showed a positive correlation with both BALF eosinophil counts ($r = 0.482$, $p = 0.031$) and the cellular infiltration score ($r = 0.760$, $p = 0.0004$), but not with serum IgE levels ($r = 0.396$, $p = 0.104$) in adult mice. In juvenile mice, the mucus cell score positively correlated only to the cellular infiltration score ($r = 0.764$, $p = 0.0006$), but neither to IgE levels ($r = 0.531$, $p = 0.0506$) nor to eosinophil counts ($r = 0.472$, $p = 0.0755$).

The result of the mucus area in each group was shown in table 2. The value was similar in the 4 groups examined.

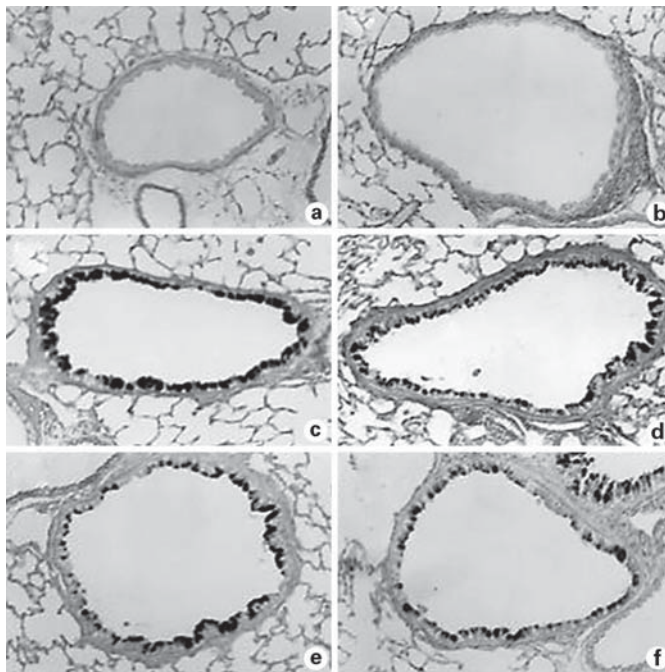


Fig. 5. Representative photomicrographs of 6- μ m sections of mouse lung (AB/PAS staining). The sections of control animals **a**: juvenile; **b**: adult animals) and those of animals sensitized and challenged with 10 (**c**: juvenile; **d**: adult animals) and 1,000 μ g of OVA (**e**: juvenile; **f**: adult animals) are shown. In OVA-challenged mice, goblet cell metaplasia (GCM) was prominent (**c–f**). Juvenile mice showed a rather obvious GCM comparing to adult ones (magnification $\times 100$).

Table 2. The mucus cell score and the mucus area

Mice	OVA dose, μ g		
	0	10	1,000
Mucus cell score			
Juvenile	0.3 $\pm$ 0.1	3.5 $\pm$ 0.8*	2.7 $\pm$ 1.1*
Adult	0.1 $\pm$ 0.1	2.7 $\pm$ 0.3*	2.6 $\pm$ 0.4*
Mucus area, %			
Juvenile	3.3 $\pm$ 1.0	42.9 $\pm$ 3.4*	36.4 $\pm$ 4.3*
Adult	0.2 $\pm$ 0.1	40.1 $\pm$ 4.8*	31.5 $\pm$ 4.1*

Values are expressed as mean $\pm$ SEM.

* $p < 0.01$ vs. the corresponding animals given vehicle for OVA (control) ($n = 8$ – 10 mice for each group.)

Relationship between BHR and Other Parameters

In adult mice, PC200 showed significant inverse correlations to the degree of the cellular infiltration score ($r = 0.554$, $p = 0.005$), eosinophil count in BALF ($r = 0.423$, $p = 0.013$), and OVA-specific IgE levels ($r = 0.547$, $p = 0.007$), but not with the mucus cell score. MaxR showed significant positive correlations to the degree of the mucus cell score ($r = 0.458$, $p = 0.037$), and IgE levels ($r = 0.600$, $p = 0.0024$) in addition to the cellular infiltration score ($r = 0.757$, $p < 0.0001$) and BALF eosinophil count ($r = 0.401$, $p = 0.011$). In juvenile mice, PC200 showed significant inverse correlations to the degree of the cellular infiltration score ($r = 0.557$, $p = 0.025$), eosinophil count in BALF ($r = 0.367$, $p = 0.094$), IgE levels ($r = 0.480$, $p = 0.038$), but not to the mucus cell score. By contrast, MaxR in juvenile mice was positively correlated with the cellular infiltration score ($r = 0.513$, $p = 0.042$) and the mucus cell score ($r = 0.556$, $p = 0.026$), but not with the BALF eosinophil count or IgE levels.

Discussion

In the present study, we have demonstrated that juvenile mice, that were sensitized since 3 days after birth and, then, repeatedly exposed to antigen, showed both systemic immune response and airway inflammation 3 weeks after the first sensitization. Sensitization with 10, but not 1,000, μ g OVA induced elevated IL-4 and decreased IFN- γ levels in BALF in both age groups, suggesting that 10 μ g OVA induced Th2-dominant conditions whereas 1,000 μ g did not. As a result, allergic airway inflammation, including BHR, eosinophilia in BALF, the cellular infiltration, GCM, and the IgE antibody production, was more prominent in mice sensitized with 10 μ g than in those sensitized with 1,000 μ g OVA in both juvenile and adult animals. Among these responses, BALF IL-4 production, GCM, and BHR were comparable between juvenile and adult mice, whereas other parameters such as IgE production, eosinophilia in BALF, inflammatory cell infiltration into airway tissues were more prominent in adult than in juvenile animals, especially, in those given 1,000 μ g OVA. It is reasonable to think that the results of the present study reflect not only the age at sensitization but also that at rechallenge with antigen.

Maturation of the immune system may be a critical element in the development of childhood asthma [14]. The immune response in the perinatal and neonatal period is thought not to be the same as that seen in mature individuals. It was shown that most infants and young

children who will go on to have persistent wheezing and asthma show high IgE production and eosinophilic immune responses at the time of their first viral lower respiratory tract illness [15, 16]. Prescott et al. [17, 18] demonstrated a continuation of fetal allergen-specific Th2 response during infancy and a decreased capacity for the production of the Th1 cytokine, IFN- γ , in those children who subsequently had atopic diseases. In experimental animals, neonatal mice are demonstrated to have Th2-biased immune responses against antigen [19, 20]. In the present study, sensitization with 10 μ g OVA induced a Th2-biased immune environment of a similar degree in juvenile and adult animals, in view of the degree of elevated IL-4 and decreased IFN- γ levels in BALF. On the other hand, sensitization with 1,000 μ g induced a Th1-biased environment compared to that with 10 μ g OVA in adult mice. In contrast to adult animals, even 1,000 μ g OVA still induced a Th2-biased immune environment in juvenile animals, although the degree was less than in those given 10 μ g OVA; that is, only a small but significant increase in BALF IL-4 level was found, and IgG2a antibody was not produced. These results confirm previous observations that neonatal mice had Th2-dominant immune responses against antigen. Previous reports investigating the effect of different sensitizing doses on the immune response indicated that higher doses of sensitizing antigen induced Th1-dominant immune responses in adult mice. In the present study, however, sensitization of 1,000 μ g OVA in adult animals showed immunological features of both Th1 and Th2 response, that is, significant amounts of OVA-specific IgE as well as IgG2a antibodies were produced in addition to a slight but significant increase in eosinophils in BALF. We do not know the reason for these results. It is possible that several factors, such as the dose of antigen or the way of sensitization/provocation procedures, were not enough to induce a Th1 response in adult animals. In addition, we do not know whether the lower dose of antigen induces a Th2-deviated response when we employ different mouse strains or different routes for sensitization, such as the intracutaneous route.

The specific IgE antibody level in the peripheral blood was more prominent in adult and juvenile mice, whereas IL-4 response in the BALF was similar or greater in juvenile animals. The results may indicate a functional immaturity of B lymphocytes or other cells that are responsible for the immune system of juvenile animals, although the details are to be elucidated. On the other hand, the degrees of BHR and GCM were comparable in juvenile and adult animals. MaxR, a parameter of BHR, was not

significantly correlated to the IgE antibody level or eosinophil counts in BALF in juvenile mice, while it was correlated to them in the case of adult animals. In addition, the degree of GCM was correlated to neither IgE levels nor eosinophil counts in juvenile mice. These results suggest a possibility that the development of BHR and GCM was not totally dependent on IgE- and eosinophil-mediated allergic airway inflammation, especially in the airways of juvenile animals. Recently, allergic sensitization with OVA has been shown to cause GCM in rodents [21], and the Th2 cytokines IL-4 and IL-13 have been implicated to be involved in this process [22]. In IL-5-knockout mice, OVA sensitization no longer leads to eosinophil recruitment after allergen exposure, but GCM still occurs [23], suggesting that eosinophils were not essential to GCM. Our present results confirm these previous observations that GCM, a feature of allergic airway inflammation, develops independently of eosinophilic inflammation. We do not know the exact reason why the degree of GCM was comparable between juvenile and adult animals, even those given 1,000 μ g OVA sensitization. In guinea pigs, we have shown that the renewal of epithelial cells was significantly more marked in juvenile and adult animals than in old ones after repeated exposure to antigen [24]. Thus, if this was the case for murine epithelial cells, it would be possible that a greater degree of epithelial cell renewal occurred in response to antigen exposure in juvenile than in adult mice. Measurements of other cytokines might be informative to analyze the reasons. Further experiments would be required to clarify the mechanism.

In the present study, it was demonstrated that GCM and, consequently, airway mucus hypersecretion might be an important component of allergic airway inflammation in juvenile mice. If this was the case in humans, the regulation of mucus hypersecretion would be beneficial not only for the treatment but also for the prevention of asthma in younger children, especially in infants. Our juvenile murine model of allergic airway inflammation may be helpful for the understanding of the pathophysiology of infantile asthma.

Acknowledgements

The authors wish to thank Mrs. Tomoko Endo, Chinori Iijima, and Shigeyo Yoshida for their technical assistance and animal care. This work was supported, in part, by the Grant-in-Aid for Scientific Research (C) (2) (No. 13670784) and (B) (2) (No. 14770568) from the Ministry of Education, Science, Sports and Culture of Japan.

References

- 1 NHLBI: Global Initiative for Asthma. Global strategy for asthma management and prevention. NIH Publication, 2002.
- 2 Gerritsen J, Koeter GH, Postma DS, Schouten JP, Knol K: Prognosis of asthma from childhood to adulthood. *Am Rev Respir Dis* 1989; 140:1235–1330.
- ▶ 3 Panhuysen CI, Vonk JM, Koeter GH, Schouten JP, van Altena R, Bleecker ER, Postma DS: Adult patients may outgrow their asthma: A 25-year follow-up study. *Am J Respir Crit Care Med* 1997;155:1267–1272.
- 4 Japanese Society of Pediatric Allergy and Clinical Immunology: Infantile asthma. In: Guidelines for the treatment and management of pediatric asthma 2002, Tokyo, Kyowa Kikaku, 2002, pp 75–98 (in Japanese).
- ▶ 5 Kay AB: Advances in immunology. Allergy and allergic diseases. First of two parts. *N Engl J Med* 2001;344:30–37.
- ▶ 6 Kay AB: Advances in immunology. Allergy and allergic diseases. Second of two parts. Allergic diseases and their treatment. *N Engl J Med* 2001;344:109–113.
- ▶ 7 Sakai K, Yokoyama A, Kohno N, Hiwada K: Effect of different sensitizing doses of antigen in a murine model of atopic asthma. *Clin Exp Immunol* 1999;118:9–15.
- ▶ 8 Hamada K, Goldsmith CA, Kobzik L: Increased airway hyperresponsiveness and inflammation in a juvenile mouse model of asthma exposed to air-pollutant aerosol. *J Toxicol Environ Health* 1999;58:129–143.
- ▶ 9 Hamelmann E, Schwarze J, Takeda A, Oshiba A, Larsen GL, Irvin C, Gelfand EW: Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Crit Care Med* 1997;156:766–775.
- ▶ 10 Curtis J, Byrd P, Warnock M, Kaltreider H: Requirement of CD4-positive T cells for cellular recruitment to the lungs of mice in response to a particulate intratracheal antigen. *J Clin Invest* 1991;88:1244–1254.
- 11 Trifilieff A, El-Hashim A, Bertrand C: Time course of inflammatory and remodeling events in a murine model of asthma: effect of steroid treatment. *Am J Physiol* 2000;279:L1120–L1128.
- ▶ 12 Lou Y-P, Takeyama K, Grattan KM, Lausier JA, Ueki IF, Agusti C, Nadel JA: Platelet-activating factor induces goblet cell hyperplasia and mucin gene expression in airways. *Am J Respir Crit Care Med* 1998;157:1927–1934.
- ▶ 13 Ohkawara Y, Lei XF, Stampfli MR, Marshall JS, Xing Z, Jordana M: Cytokine and eosinophil responses in the lung, peripheral blood, and bone marrow compartments in a murine model of allergen-induced airways inflammation. *Am J Respir Cell Mol Biol* 1997;16:510–520.
- ▶ 14 Martinez F: Maturation of immune responses at the beginning of asthma. *J Allergy Clin Immunol* 1999;103:355–361.
- ▶ 15 Marguet C, Jouen-Boedes F, Dean T, Warner J: Bronchoalveolar cell profiles in children with asthma, infantile wheeze, chronic cough or cystic fibrosis. *Am J Respir Crit Care Med* 1999; 159:1533–1540.
- ▶ 16 Ferguson AC, Whitelaw M, Brown H: Correlation of bronchial eosinophil and mast cell activation with bronchial hyperresponsiveness in children with asthma. *J Allergy Clin Immunol* 1992;90:609–613.
- ▶ 17 Prescott S, Macaubas C, Smallacombe T, Holt B, Sly P, Holt P: Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999;353:196–200.
- ▶ 18 Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD, Holt PG: 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.
- ▶ 19 Forsthuber TYH, Lehmann PV: Induction of TH1 and TH2 immunity in neonatal mice. *Science* 1996;271:1728–1730.
- ▶ 20 Adkins B, Du RQ: Newborn mice develop balanced Th1/Th2 primary effector responses in vivo but are biased to Th2 secondary responses. *J Immunol* 1998;160:4217–4224.
- ▶ 21 Blyth DI, Pedrick MS, Savage TJ, Hessel EM, Fattah D: Lung inflammation and epithelial changes in a murine model of atopic asthma. *Am J Respir Cell Mol Biol* 1996;14:425–438.
- ▶ 22 Grunig G, Warnock M, Wakil A, Venkayya R, Brombacher F, Rennick DM, Sheppard D, Mohrs M, Donaldson DD, Locksley RM, Corry DB: Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1999; 282:2261–2263.
- ▶ 23 Cohn L, Homer RJ, MacLeod H, Mohrs M, Brombacher F, Bottomly K: Th2-induced airway mucus production is dependent on IL-4Ra, but not on eosinophils. *J Immunol* 1999; 162:6178–6183.
- ▶ 24 Ohki Y, Tokuyama K, Sato A, Nishimura H, Tabata M, Tomiyoshi K, Inoue T, Arakawa H, Kato M, Mochizuki H, Morikawa A: Maturation changes in airway remodeling after chronic exposure to ovalbumin in sensitized guinea pigs: Role of the cell renewal of airway resident cells. *Pediatr Res* 2002;52:525–532.