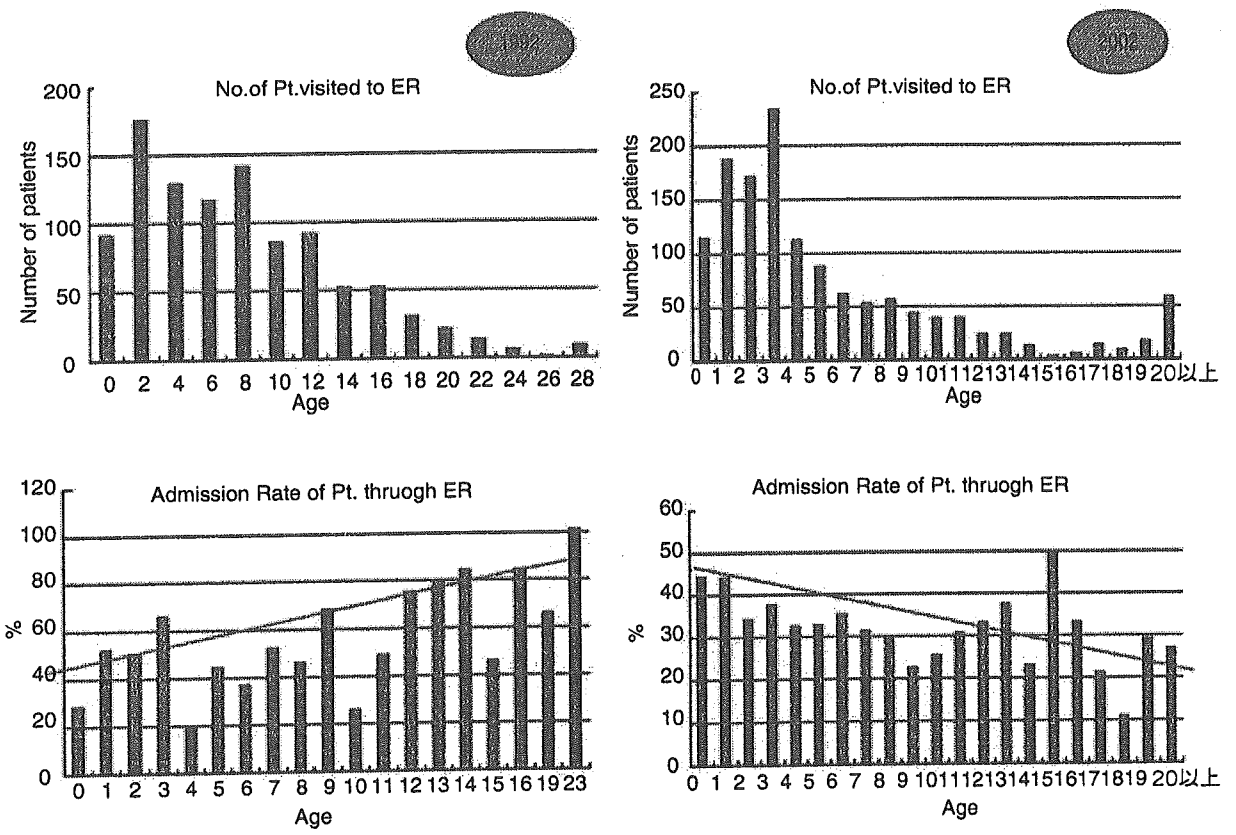

Fig. 4 Number of patients who visited the ER and admission rate

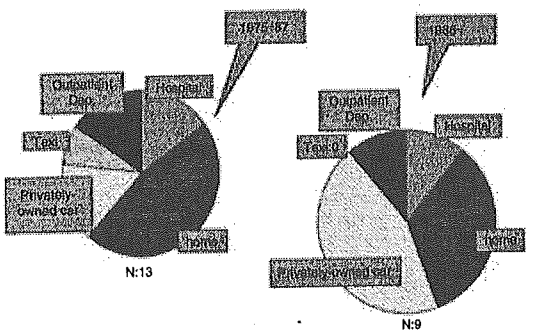

Fig. 2 Place of death

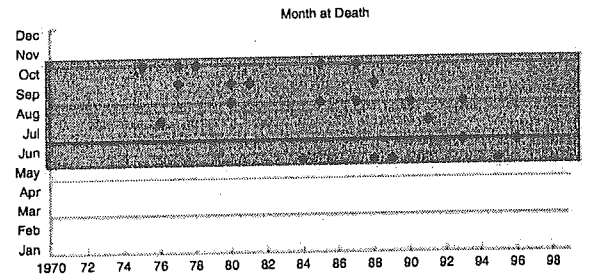

Fig. 3 Calender months in which fatalities ocvurred

In 1992, there was a difference in the number of patients visiting the ER and those who were subsequently hospitalized. Namely in 1992, the patients in puberty visiting to ER had a severe attack to be hospitalized. There was no such a tendency in 2002.

Figure 5 shows the age distribution of acutely hospitalized asthmatic patients in 1977 (a), 1987 (b), and 1997 (c), in the Department of Pediatrics, National Fukuoka Hospital, with (□) or without (■) concomitant infection.[3] These data suggest that in 1992 patients in puberty were not controlled adequately. This conclusion was also indicated by the data from the study about the near-fatal cases mentioned below.

CLINICAL SIGNIFICANCE OF NEAR-FATAL CASES IN OUR HOSPITAL

In this paper, a near-fatal case means a rescued case that could not have been rescued if treatment had not been given early enough or fatal cases that could have been prevented had treatment been given early enough. Figure 6 shows the age distribution of the fatal cases (reported to the Japanese Society of Pediatric Allergy and Clinical Immunology (upper figure)) and near-fatal cases treated in our hospital. Fatal cases peaked in puberty, but the peak of near-death

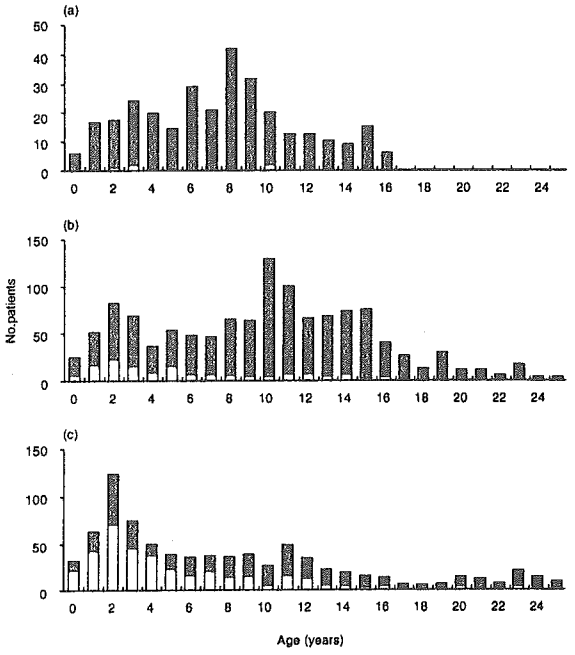

Fig. 5 Age distribution of acutely hospitalized asthmatics in (**a**) 1977, (**b**) 1987 and 1977 (**c**), Division of Pediatrics, National Minami Fukuoka Chest Hospital, with (□) or without (▨) concomitant infection.

cases was in puberty, infancy and early childhood. This finding suggests that there are more dangerous factors in puberty than in infancy or early childhood.

Figure 7 shows the prescription rate of theophylline, ICS, and beta-stimulants. The prescription rate was calculated as follows : (number of days of use indicated by the calculation of the number of puffs of the prescribed drug/number of days on which this drug should be used in a year) ×100. The ideal result should be 100%.

As shown in Figure 7, ICS observed low level of compliance in patients aged 15 or more who had regularly visited our out patient department (OPD). A failure of management, often observed among young patients, may be one of the important factors for the mortality in asthma.[4]

Figure 8 indicates the responses to the question "when do you visit the OPD?", and shows that patients in puberty do not visit the OPD regularly, especially in boys.

Figure 9 shows that the patients in puberty had many inhalations during attacks before visiting the OPD. This tendency might delay the beginning of the next step of treatment for severe attacks.

When asked if patients have someone who can take them to the hospital when they have attacks male patients were found to have nobody(Fig. 10).

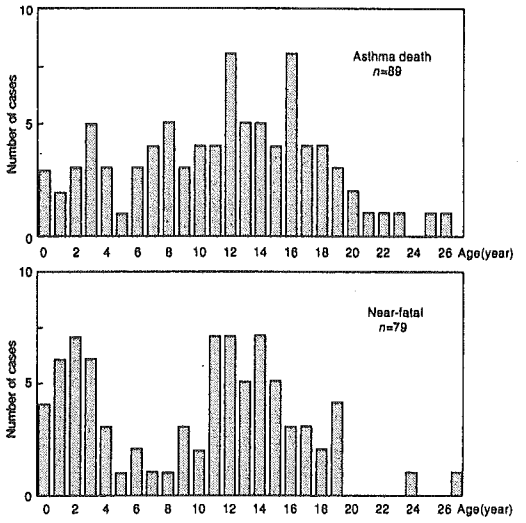

Fig. 6 Age distribution of asthma death and near-fatal cases

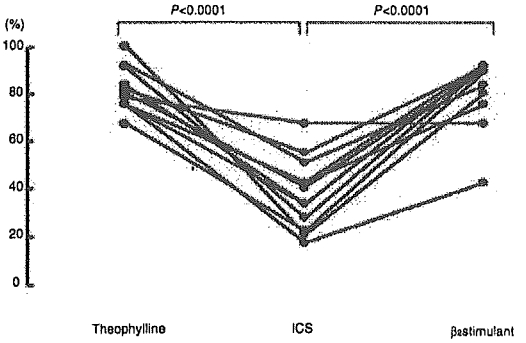

Fig. 7 Prescription Rate of theophylline, inhaled corticosteroid(ICS), and β_2 stimulant in patients aged 15 years or more with regular use of ICS

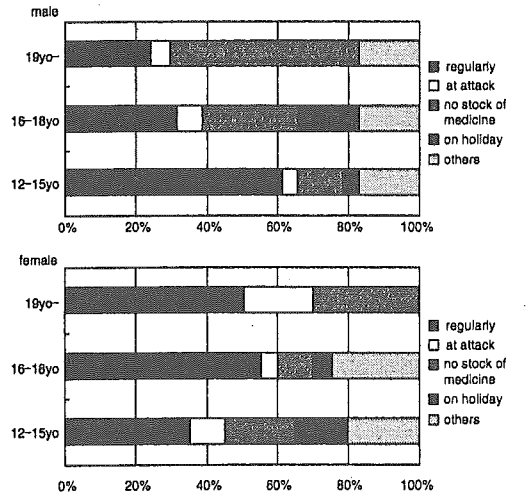

Fig. 8 When do you visit OPD?

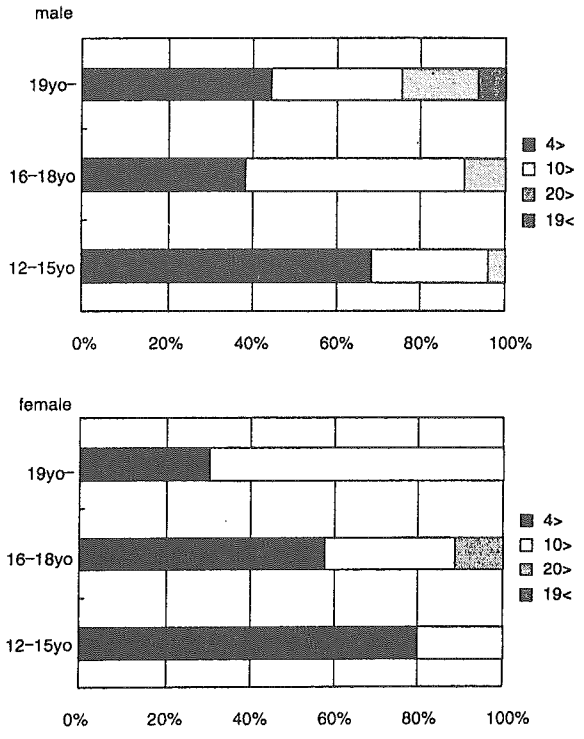

Fig. 9 How many times do you use the inhaler on severe attacks?

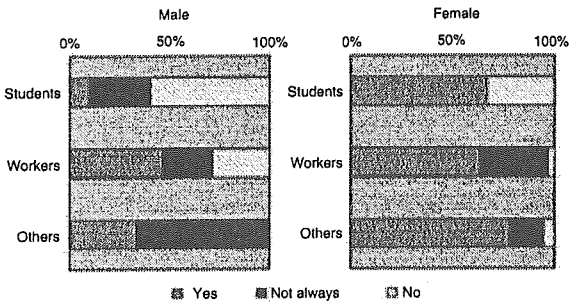

Fig. 10 "Do you have someone who can take you to the hospital?" (Subjects : patients with history of hospitalization, 20–30 years old)

These data provide some reasons why male asthmatic patients are at higher risk of asthma death than female.[5]

CASE OF NEAR-FATAL DEATH

CASE 1 : 10-YEAR-OLD BOY

One month before our summer camp, for which he had applied, his %FVC was 50%. However, both the patient and his mother said that his condition was normal.

On the first day of camp, severe dyspnea developed and he suffered respiratory arrested. He was rescued by ventilation, intravenous drip, sulbutamol, hydrocortisone, and aminophylline. We continued intravenous drip for 4 days then the patient was admitted to our hospital after camp.

After one year of drug therapy and exercise, his lung function became normal and he was discharged. Currently, the patient is in good condition and visits his local doctor several times per year.

CASE 2 : 14-YEAR-OLD GIRL

The patient was hospitalized for 1 year when she was in third grade in elementary school. She was asymptomatic after entering junior high school, but symptoms returned when she discontinued complementary medicine that she started for atopic dermatitis.

The patient was given DSCG+β-agonist which she used every hour (3 times more frequently than instructed) when she had attacks. The patients developed severe dyspnea and was brought to our clinic by ambulance, with mouth to mouth artificial respiration by her father. Although she was rescued, discontinuation of inhalation therapy quickly worsened the condition. β-agonist dependence, β-blockade condition was suspected. It took 3 months for her to recover from this condition.

CASE 3 : 18-YEAR-OLD BOY

The patient had had severe asthma since he was in elementary school and was treated by a local hospital. While he was playing a video game, he had an asthma attack and used his meterd-dose inhaler (MDI) in several times. After a while he asked his sister to call an ambulance.

When she returned from phone, he had respiratory arrest, with the MDI in his mouth. After he had entered high school, oral steroids and MDI had been prescribed by a local doctor. Drug compliance was poor. Underestimation of severity was suspected.

CASE 4 : 17-YEAR-OLD BOY

One day, the patient told his teacher he would go home early. His family called that night saying that the patient was not home yet. Next morning, the patient was found dead in the school toilet room, with his MDI in his mouth. Neither teachers nor friends knew that he had asthma. If he had not hidden his condition and used the MDI in the classroom, he might have been saved.

DISCUSSION ABOUT PROBLEMS IN ABOVE CASES

In case 1, the patient's family underestimated the severity. Since dyspnea is a subjective feeling and becomes severe gradually, it is difficult to estimate the severity. The family did not understand that dyspnea must be treated immediately.

In Case 2, the patient's family said the asthma was well controlled. Although there are many asthma

Table 2 Causes of near-fatality

Ⅰ. Problems with the whole disease
1. Disease itself is severe
① β-blockade
② Adrenocortical insufficiency
③ Airway hypersensitivity
2. Above conditions falsely classified as mild

Ⅱ. Medical problems such as diagnosis and treatment
1. Underestimation of severity (patients and their family)
① They thought that attacks had to appear "violent"
② They are used to attacks, as they experienced many attacks
③ Insufficient knowledge about attacks
2. Delay in visiting hospital when patients have attacks
① Underestimation of severity of attacks
② MDI over-use
③ The feeling that they can contain the symptoms since they were able to last time
④ They did not use an ambulance
3. Inadequate usage of drug
① Over-use for attacks (do not proceed to the next step when they should)
They think they can contain it (Fig. 1)
② Under-use for attacks (do not use when they should)
i) Underestimation of severity
ii) Severity is not recognized by the doctor
iii) Not enough drugs (irregular visits)
iv) Concern about others in their surroundings make them miss the proper timing for taking drugs
③ Under-use of preventive drugs
i) No drug
ii) Forgot
iii) Care about others in their surroundings and miss the proper timing for taking drugs

Ⅲ. Psychological, sociological, and economical factors
1. Too busy, working too hard
① Working too hard at work or school and delay in treatment
2. Delay in primary treatment
① Nobody is around when patients have attacks (living alone)
② They do not want to take medicines in front of other people (hiding the conditions)

Ⅳ. Others

death reports concerning mild asthmatic patients, the severity might have been underestimated in this case. Complementary medicine can be dangerous. It has been known for a long time that some patients acquire resistance to β-agonists. This patient would have dead if her father had not practiced artificial respiration, suggesting the importance of early action.[6-9]

In Case 3, poor drug compliance during adolescence and insufficient follow up after discharge were the problems. The patient underestimated the severity and tried to contain the symptoms with MDI. Since symptoms improve, at least slightly, immediately after inhalation, it is difficult for patients to know when to visit to emergency services (Fig. 11).

This is a danger of inhalation therapy. Over-use of β-stimulant should be very important factor for the mortality.[10,11] As in case 2, symptoms suddenly worsen.[12]

Case 4 suggests problems about hiding the disease.

Table 2 summarizes these problems. The suspected reasons why patients do frequent inhalation and delay visiting the ER are shown in Figure 11.

Therefore, it is important to teach them how dangerous the frequent use of MDI is, and to make the OPD have an atmosphere easy to visit for patients.

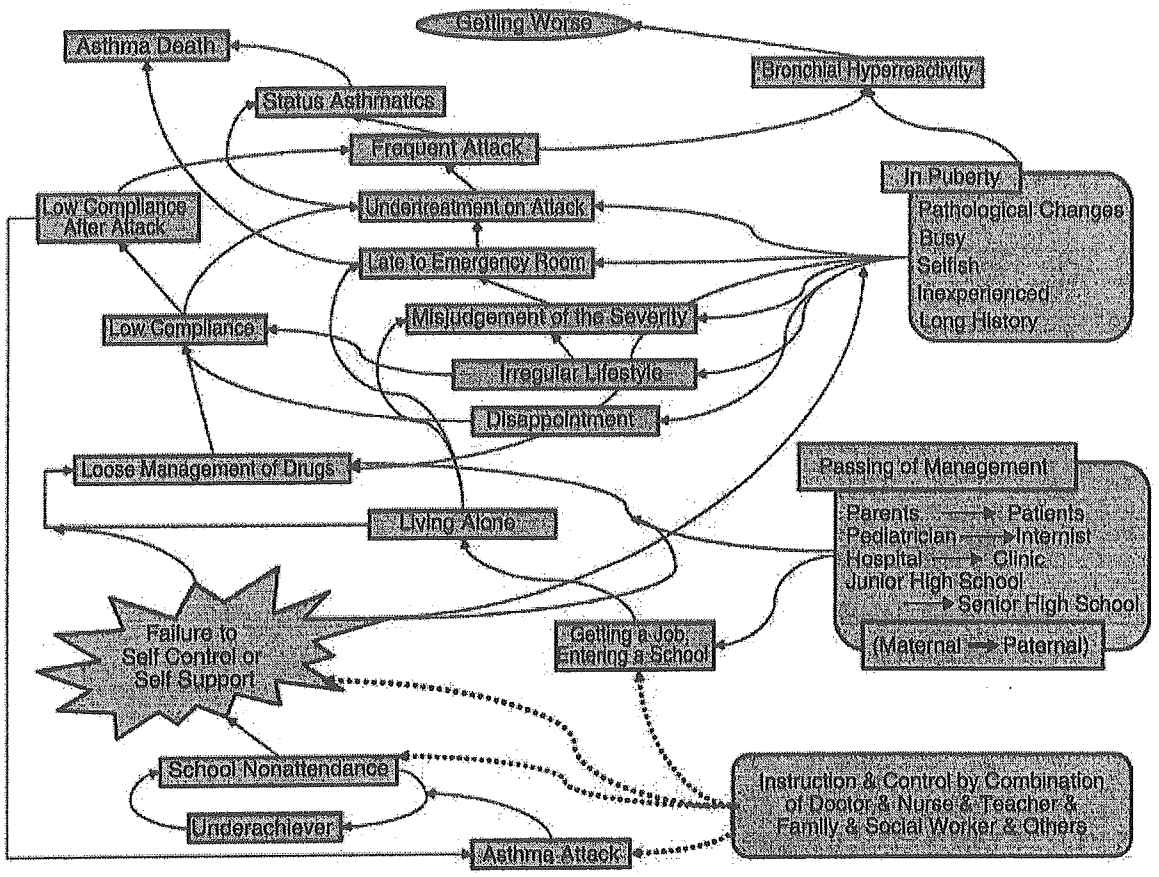

Fig. 12 Problems of adolescent asthma.

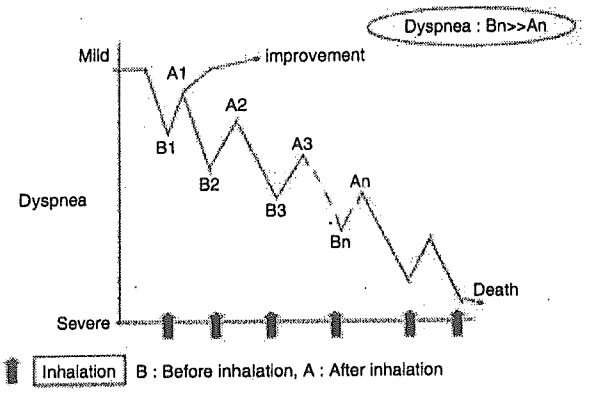

Fig. 11 Dyspnea before and after inhalation of β-agonist (Why do they think they can continue to use its.) After any time of inhalation, the severity of dyspnea before inhalation is stronger than that after inhalation.

We show a list of required actions and cautions in Table 3. Almost all fatal cases were in puberty or elder by age. In adolescents, many sociological/economical/educational problems are present (Fig. 12).[3] We should take action and be cautions before puberty to prevent and resolve these problems, if even a little.

CONCLUSION

In early childhood and puberty, asthmatic patients are at high risk of near-fatal asthma, and, in puberty, at high risk of asthma death.

We conclude the clinical importance of the following in order to decrease asthma deaths :

(1) In puberty, improvement of drug compliance, adequate estimation of severity of asthmatic attack, and eliminate delays in visiting hospital.

Good relationship between patients and society, not to hide his asthma.

(2) Early childhood is high risk age for asthmatic death.

Families of patients in early childhood have little experience or ability to evaluate the severity of asthmatic attack or when to visit the ER.

Table 3 Treatment for near-fatal cases

1. Acute attacks
   - ① Administer sufficient oxygen and treat for status asthmaticus
     {Continuous inhalation of isoproterenol should be performed at high concentration with sufficient oxygen (watch for cardiac complications)}.
   - ② Admit the patients even when the symptoms appear to have subsided
   - ③ Consider near-fatal attack experiences to be good chances for reviewing previous asthma treatment and patient education, and inform the patients and their families that treatment and education will be reconsidered

2. After patients have been rescued from severe condition
   - ① Evaluate dependence on drugs (if they are dependent on drug, slowly decrease doses)
     - i) β-agonists
     - ii) Steroids
     - iii) Others
   - ② Evaluate drug compliance
     - i) Evaluate reasons why low-compliance in bad case
     - ii) Review drugs in good case
   - ③ Evaluate drug allergy

3. After patients have recovered from attacks(when the patients do not have attacks)
   - ① Review treatment plans
     - i) Review patient/family education
       (especially understanding concerning severity of attacks and understanding attacks must be prevented)
     - ii) Review lifestyle. When sociological, economical, and psychological factors are involved, those factors should be reviewed as well
       (Have someone who can take the patients to the hospital. Perform after estimation if understanding about the disease can be obtained at school or workplace)
     - iii) Review drug therapy
   - ② Review pathology
     - i) Re-test for allergens, evaluate severity of allergy
     - ii) Evaluate airway hypersensitivity, exercise-induced asthma
   - ③ Consider long-term hospitalization
   - ④ Follow-up after discharge
   - ⑤ Confirm relationship between hospital-general practitioner, doctor-family, school-teacher, work office

4. Others
   Have someone who can perform artificial respiration among family members.
   Make it possible to perform cardiopulmonary resuscitation in ambulance

REFERENCES

1. Tanihara S, Nakamura Y, Matui T, Nishima S. A case-control study of asthma death and life-threatening attack : Their possible relationship with prescribed drug therapy in Japan. *J. Epidemiol.* 2002;**12**:223-228.
2. Adachi M, Morikawa A, Ishihara K. Asthma Insights & Reality in Japan. *Jpn. J. Allergol.* 2002;**51**:411-420.
3. Nishima S. Treatment and managemaent of severe asthma in childhood through to young adult patients : 12, Japanese Society of Allergology Meeting, Spring Clinical Meeting, Lecture of the Chairman. *Allergol. Int.* 2001;**50**: 249-264.
4. NIH/ NHLBI. *Global Initiative for Asthma.* 2003;16-17.
5. Odajima H. Importance of self-support for asthma control in adolescents. In : Sinomiya K (ed.) . *Current advances in pediatric allergy and clinical immunology.* Osaka:Churchill Livingstone, 1996;11-14.
6. Crago S, Coors L, Lapidus JA, SApien R, Murphy SJ. Prehospital treatment of acute asthma in rural state. *Ann. Allergy Asthma Immunol.* 1998;**81**:322-325.
7. Simpson AJ, Matusiewics SP, Brown PH *et al.* Emergency prehospital management of patients admitted with acute asthma. *Thorax* 2000;**55**:97-101.
8. Strunk RC, Nicklas RA, Milgrom H, Ikle D. Fatal and near-fatal asthma questionnaire ; prelude to a national registry. *J. Allergy Clin. Immunol.* 1999;**104**:763-768.
9. Sapien RE, Lapidus J, Coors L, Murphy SJ. Prehospital EMS treatment of pediatric asthma and what happens before help arrives? *J. Asthma* 1997;**34**:477-481.
10. Crane J, Pearce N, Flatt A, Burgess C, Jackson R, Kwong T. Prescribed fenoterol and death from asthma in New Zealand, 1981-83 : case-control study. *Lancet* 1989;**1**:917-

922.
11. Spizer WO, Suisa S, Ernst P, Horwitz RI, Habbick B, Cockcroft D. The use of beta-agonists and the risk of death and near death from asthma. *N. Eng. J. Med.* 1992; **326**:501-506.
12. Thomas SD, Whitman S. Asthma hospitalization and mortality in Chicago : an epidemiologic overview. *Chest* 1999;**116**:135S-141S.
13. Odajima H. Current insight of adolescent asthma. *Jpn. J. Allergol.* 2000;**49**:459-462.

Pediatr Allergy Immunol 2005: 16: 354–356

PEDIATRIC ALLERGY AND IMMUNOLOGY
DOI: 10.1111/j.1399-3038.2005.00283.x

Short Communication

Fetal growth promotion in allergic children

Kawano Y, Morikawa M, Watanabe M, Ohshiba A, Noma T, Odajima H. Fetal growth promotion in allergic children.
Pediatr Allergy Immunol 2005: 16: 354–356. © 2005 Blackwell Munksgaard

Several *in vitro* studies have suggested the presence of Th2-skewed immunity during pregnancy in infants with atopic diseases. Our study indicated that allergic infants showed a higher birth weight and shorter gestational period at birth than those of non-allergic peers. Moreover, allergic mothers gave birth to neonates whose birth weights and gestational ages were higher and shorter than those of the non-allergic mothers, respectively. Thus, our data clearly demonstrated the promotion of intrauterine growth, either in the allergic children, or allergic mothers. Such an intrauterine environment favorable for the fetal growth may also accelerate the development of allergic diseases in their offspring that are most probably caused by the Th2-oriented immunity.

Yutaka Kawano[1], Miki Morikawa[2], Misa Watanabe[3], Akihiro Ohshiba[4], Takeshi Noma[5] and Hiroshi Odajima[6]

[1]Department of Pediatrics, Yokohama City Minato Red Cross Hospital, Yokohama, Japan, [2]Department of Pediatrics, JR Sendai Hospital, Sendai, Japan, [3]Department of Pediatrics, Toho University, Ohmori Hospital, Tokyo, Japan, [4]Department of Pediatrics, Tokyo Medical and Dental University, Tokyo, Japan, [5]Department of Pediatrics, Kitasato University, Sagamihara, Japan, [6]Department of Pediatrics, Fukuoka National Hospital, Fukuoka, Japan

Key words: fetal growth; birth weight; gestational age; development of allergy; atopic dermatitis; bronchial asthma; Th2

Yutaka Kawano, Department of Pediatrics, Yokohama City Minato Red Cross Hospital, 3-12-1 Shin-Yamashita, Naka-ku, Yokohama, Kanagawa 231-8682, Japan
Tel: +81-45-628-6100
Fax: +81-45-628-6101
E-mail: kawanoyutaka@mbj.ocn.ne.jp

Accepted 14 March 2005

Successful pregnancy requires a maternal immunity biased toward Th2 rather than Th1 (1). Several *in vitro* studies have presented evidence of a Th2-skewed immune response in the atopic children, coming as early as the moment of birth itself (2). A delayed maturation of the Th1 response may lead to the development of the allergic diseases in the infants during the first 2 yr of life (3). However, clinical data that support such an increased bias toward Th2 in the potentially allergic newborns is still lacking.

About 279 children of 1 yr old, including 142 males and 137 females, who visited the pediatric clinics at one of our hospitals in Japan asking for requesting medical care or for a health examination were enrolled into this study. Any controls, who, are attending due to any condition that is linked to low birth weight were excluded. Data on birth weight and gestational age was obtained from the medical record. The mothers of the children, that were eligible for the detailed questionnaire modified from that provided by the International Study of Asthma and Allergies in Childhood (ISAAC) (4) were asked about the birth conditions reflecting the intrauterine period of life. Informed consent was also obtained from the mothers.

Based on the questionnaire and physical examinations, the presence or absence of maternal allergic diseases was determined. Allergic mothers were defined as having at least one of the five major allergic diseases, including bronchial asthma, atopic dermatitis, allergic rhinitis, Japanese pollinosis and allergic conjunctivitis, whereas mothers having with none of the above these five allergic disorders were diagnosed as non-allergics.

The diagnosis of atopic dermatitis was based on a physical examination by pediatric allergologists according to the Hannifin and Rajka criteria (5). Typical morphology and distribution such as facial and extensor involvement and chronic or chronically relapsing dermatitis were two important criteria. The other two factors including onset at an early age and a personal or family history of atopy were omitted, because

they were not useful for young children in this study. Similarly, children having experienced recurrent wheezing episodes confirmed by medical records were diagnosed as having asthmatic by pediatric allergologists, based on the criteria of the American Thoracic Society (6). Of the 279 children in the present study, 50 (18.0%) had atopic dermatitis and 52 (18.6%) had bronchial asthma. About 20 (7.2%) had both of them. Mann–Whitney *U*-test was used to analyze the data.

As shown in Table 1, the birth weights of the allergic children, having either atopic dermatitis or bronchial asthma, were significantly higher than those of non-allergics and the gestational period at birth of the allergic children was also significantly shorter than those that of non-allergics (Mann–Whitney *U*-test; $p < 0.001$). Similarly, children with atopic dermatitis showed higher birth weights and shorter gestational ages than those without this condition, while the presence or absence of bronchial asthma also revealed the same trends (Mann–Whitney *U*-test; $p < 0.001$).

In addition to the presence or absence of the infantile allergic diseases, the allergic status of their mothers may affect the development of allergy in their offspring. Thus, allergic mothers gave birth to neonates whose birth weights and gestational ages were higher and shorter than those of the non-allergic mothers, respectively, as in Table 2 (Mann–Whitney *U*-test; $p < 0.001$).

To confirm the data of Mann–Whitney *U*-tests, logistic regression analysis was constructed by using birth weight, gestational age, infantile gender, maternal infection during pregnancy and respiratory infections early in infancy. The results of the logistic regression analysis indicated that birth weight increased the risk of allergy and atopic dermatitis, but not that of bronchial asthma.

Previous studies have indicated an association between the development of atopic diseases and the birth weight and the gestational age (7–10).

Table 1. Comparisons of the weights and gestational periods at birth between allergic and non-allergic children

Status of the children	Birth weight (g)	Gestational period (wk)
Allergy (+)	3040.4 ± 441 (n = 82)*	38.76 ± 1.71 (n = 68)*
Allergy (−)	2891.5 ± 418 (n = 190)	39.48 ± 7.10 (n = 157)
Atopic dermatitis (+)	3095.2 ± 389 (n = 50)*	39.17 ± 1.41 (n = 42)*
Atopic dermatitis (−)	2900.6 ± 431 (n = 222)	39.29 ± 6.63 (n = 183)
Bronchial asthma (+)	3012.5 ± 455 (n = 52)*	38.47 ± 1.97 (n = 43)*
Bronchial asthma (−)	2918.4 ± 422 (n = 220)	39.46 ± 6.60 (n = 182)

Data represent mean ± s.d.
*Mann–Whitney *U*-test $p < 0.001$.

Table 2. Comparisons of the weights and gestational periods at birth between neonates born to allergic and non-allergic mothers

Status of the mother	Birth weight (g)	Gestational period (wk)
Allergy (+)	2949.64 ± 353.143 (n = 98)*	38.7326 ± 3.4791 (n = 86)*
Allergy (−)	2928.97 ± 468.102 (n = 174)	39.3857 ± 7.53179 (n = 140)

Data represent mean ± s.d.
*Mann–Whitney *U*-test $p < 0.001$.

However, our data did not correlate with the study by Olesen et al., who demonstrated that a high birth weight and high gestational age were associated with an increased risk of atopic dermatitis (7). Moreover, another alternative research indicated that the risk for adult asthma is partly established early in life and suggests that poor intrauterine growth is involved in the etiology of asthma (8). The reasons for the discrepancy between their results and ours might be result from the difference in the age of the subjects involved.

In contrast, the results shown here are consistent with the data of the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study (10), that indicated a high birth weight increase was associated with a risk of atopic dermatitis in the first year of life for infants born to mothers with a respiratory allergy and with the study by Yuan et al. (9) showing that fetal growth was associated with childhood asthma. Unlike those researches, our subjects included not only allergic mothers, but also healthy mothers and our study analyzed two representative allergic diseases: namely atopic dermatitis and bronchial asthma. Moreover, the other study focused on the birth weight and gestational age outside the normal range and they concluded that babies outside normal birth weight or gestational ages, seemingly the result of abnormal pregnancies, were at risk of allergy. However, our study targeted infants born with a normal range of their birth weight and of their gestational age, which suggests that some factors promote the development of childhood allergy during a normal pregnancy. The difference in birth weights and the gestational ages between allergics and non-allergics in the present research was small and within the normal range, but it includes statistically significant differences such as $p < 0.001$.

Although factors other than birth weight and gestational age may affect the development of allergy in their children, the gender of the infant, maternal age, use of antibiotics, breast feeding, environmental smoking, pet exposure or daycare attendance were not found to be associated with

allergy. Infantile respiratory infection during infancy was associated with the development of bronchial asthma, in contrast to maternal infections during pregnancy that did not affect any allergic diseases (data not shown).

Since the atopic dermatitis and bronchial asthma conditions were independently associated with increased fetal growth promotion, it is highly likely that the development of allergic disease and a feto-maternal interface favoring fetal growth are closely linked, although our data must be regarded as preliminary given the size of the study. Nevertheless, these results strongly suggest that the exceedingly Th2-oriented feto-placental environment present in allergic individuals also promotes fetal growth.

Acknowledgments

This study was partly supported by Research on Allergic Diseases and Immunology of Health and Labour Sciences Research Grants in Japan.

References

1. Dealtry GB, O'Farrell MK, Fernandez N. The Th2 cytokine environment of the placenta. Int Arch Allergy Immunol 2000: 123: 107–19.
2. Gabrielsson S, Soderlund A, Nilsson C, Lilja G, Nordlund M, Troye-Blomberg M. Influence of atopic heredity on IL-4-, IL-12- and IFN-gamma-producing cells in in vitro activated cord blood mononuclear cells. Clin Exp Immunol 2001: 126: 390–6.
3. Bjorksten B. The intrauterine and postnatal environments. J Allergy Clin Immunol 1999: 104: 1119–27.
4. Worldwide Variations. In The Prevalence Of Asthma Symptoms: The International Study Of Asthma And Allergies In Childhood (ISAAC). Eur Respir J 1998: 12: 315–35.
5. Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol 1980: 92: 44–7.
6. Standards for the Diagnosis and Care of Patients with Chronic Obstructive Pulmonary Disease (COPD) and Asthma. This official statement of the American Thoracic Society was adopted by the ATS board of Directors, November 1986. Am Rev Respir Dis 1987: 136: 225–44.
7. Olesen AB, Ellingsen AR, Olesen H, Juul S, Thestrup-Pedersen K. Atopic dermatitis and birth factors: historical follow up by record linkage. BMJ 1997: 314: 1003–8.
8. Svanes C, Omenaas E, Heuch JM, Irgens LM, Gulsvik A. Birth characteristics and asthma symptoms in young adults: Results from a population-based cohort study in Norway. Eur Respir J 1998: 12: 1366–70.
9. Yuan W, Basso O, Sorensen HT, Olsen J. Fetal growth and hospitalization with asthma during early childhood: a follow-up study in Denmark. Int J Epidemiol 2002: 31: 1240–5.
10. Kerkhof M, Koopman LP, van Strien RT, et al. Risk factors for atopic dermatitis in infants at high risk of allergy: the PIAMA study. Clin Exp Allergy 2003: 33: 1336–41.

ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (2005) 23: 1-6

A Study of the Factors Responsible for the Development of Allergic Diseases in Early Life

Yutaka Kawano[1], Miki Morikawa[2], Misa Watanabe[3], Akihiro Ohshiba[4], Takeshi Noma[5] and Hiroshi Odajima[6]

SUMMARY In order to analyze the determinants involved in the development of allergic diseases early in infancy, we examined the environmental and genetic factors that might affect the induction of such diseases during infancy, using a questionnaire. Maternal pharyngitis during pregnancy was significantly related to the development of atopic dermatitis in their progeny. Moreover, the frequency of the maternal infection was associated with a significantly increased risk of allergy in their infants. The prevalence of post-delivery maternal allergy was positively linked to the allergic symptoms in their children while the likelihood of bearing allergic children was related to the numbers of allergic individuals within their family. These results suggested that pre- and post-natal maternal factors and any genetic predisposition might modify the development of allergy in infancy.

The increased prevalence of allergic diseases in recent years may be the result of alterations in lifestyle and the reduced exposure to infectious diseases during infancy as suggested by the hygiene hypothesis.[1] In addition to the factors relating to early infancy, prenatal maternal factors may also affect the development of allergies in an offspring as has been suggested by the presence of allergen-sensitized T lymphocytes as early as 22 weeks into the gestation period.[2] The present study was performed to illustrate the factors relating to the development of allergies during the initial two years of infancy based on a questionnaire covering pre- and post-natal history and environmental factors.

MATERIALS AND METHODS

Subjects

This study included 207 children consisting of 108 males and 99 females, aged one to two years, who visited the pediatric clinics at the Yokohama Red Cross Hospital, JR Sendai Hospital, Toho University Ohmori Hospital, Tokyo Medical and Dental University, Kitasato University, and National Minami-Fukuoka Chest Hospital in Japan. Of these 207 children, 45 (21.7%) had atopic dermatitis and 22 (10.6%) had bronchial asthma. Ten (4.8%) had both of them.

Methods

Questionnaire

The mothers, that were capable of under-

From the [1]Department of Pediatrics, Yokohama City Minato Red Cross Hospital, Yokohama, Japan, [2]Department of Pediatrics, JR Sendai Hospital, Sendai, Japan, [3]Department of Pediatrics, Toho University Ohmori Hospital, Tokyo, Japan, [4]Department of Pediatrics, Tokyo Medical and Dental University, Tokyo, [5]Department of Pediatrics, Kitasato University, Sagamihara, Japan, [6]Department of Pediatrics, Fukuoka National Hospital, Fukuoka, Japan.
Correspondence: Yutaka Kawano
E-mail: kawanoyutaka@mbj.ocn.ne.jp

standing and answering the detailed questionnaire from the International Study of Asthma and Allergies in Childhood (ISAAC)[3] and the U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis,[4] were asked about their maternal history both during the pregnancy and child-bearing periods, any childhood history of diseases, vaccinations, feeding, as well as about the environment of the children, and any family history of allergic diseases. We used the ISAAC 2002 with only slight modifications especially for background elements of the environmental factors.

Diagnosis of the childhood allergic diseases

The diagnosis of atopic dermatitis was made by physical examination by a pediatrician according to the Hanifin and Rajka criteria.[5] Typical morphology and distribution such as facial and extensor involvement and chronic or chronically relapsing dermatitis were two important criteria. Two other factors, early age of onset and personal or family history of atopy, were omitted, because they were not useful for young children in this study. Similarly, children experiencing recurrent wheezing episodes confirmed by a medical record were diagnosed as having asthma by a pediatrician based on the criteria of the American Thoracic Society.[6]

Statistical analysis

A comparison of the factors that may be responsible for the development of allergy was made between allergic and non-allergic children using χ^2 test with Yates' correction (when necessary), and Spearmann correlation coefficients as appropriate. Logistic regression analysis was performed to select infectious symptoms during pregnancy significantly associated with the development of allergy. All p values less than 0.05 were defined as significant.

RESULTS

Influence of maternal infections during pregnancy on the development of allergy in their infants

Among the infectious symptoms of the mothers during pregnancy, maternal fever was significantly associated with allergic symptoms in their children that consisted of atopic dermatitis and asthma (Chi = 4.3878, $p < 0.05$). Similarly, maternal cough was associated with the development of asthma (Yates Chi = 5.9107, $p < 0.05$). However, in both cases, logistic regression analysis revealed that there were no significant associations between them. Therefore we concluded that they showed weak relevancy. Maternal pharyngitis was associated with the development of atopic dermatitis (Yates Chi = 4.417, $p < 0.05$) (Fig. 1) and this was confirmed by the results of the logistic regression analysis ($p < 0.05$). In the questionnaire, we recorded the time of the appearance of the respective symptoms, but it showed no significant association with the childhood allergy.

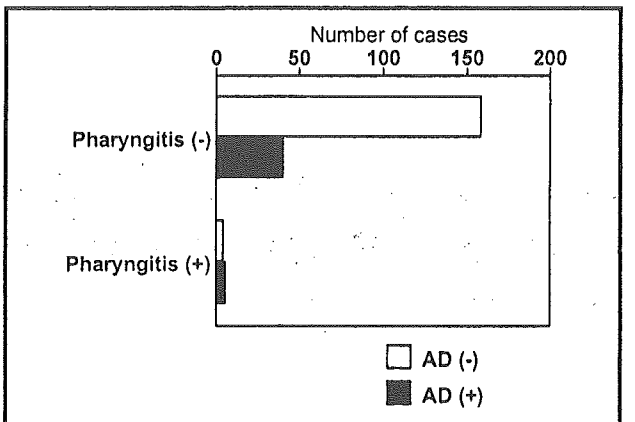

Fig. 1 Maternal pharyngitis during pregnancy and the development of atopic dermatitis in their infants. AD, atopic dermatitis.

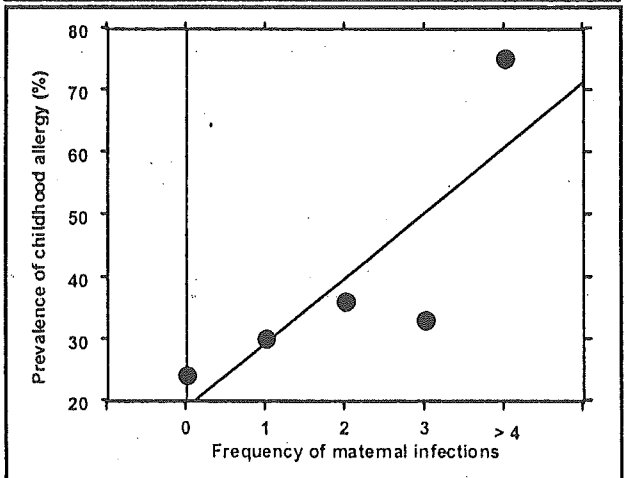

Fig. 2 A correlation between maternal infections during pregnancy and the prevalence of allergic diseases in the offspring.

Furthermore, the frequency of the maternal infections, including diarrhea, fever, rhinorrhea, cough, pharyngitis, bronchitis and pneumonia, during pregnancy closely paralleled the prevalence of allergic diseases in the offspring (r = 0.82299, p < 0.01) (Fig. 2).

Relationship between maternal allergy during pregnancy and the development of allergy in their offspring

The childhood incidence of allergic diseases, including both atopic dermatitis and asthma, differed significantly based on the allergic symptoms of their mothers during pregnancy (Yates Chi = 49.6, p < 0.01). Furthermore, childhood allergy prevailed as the number of maternal allergic symptoms increased with a strong link between them (rs = 0.955921, p < 0.01). Specifically, the incidence of atopic dermatitis in isolation correlated with these symptoms (rs = 0.955921, p < 0.01), rather than asthma in isolation (Fig. 3). In this analysis, the numbers of maternal allergic symptoms were defined as the numbers of the allergic diseases consisting of bronchial asthma, atopic dermatitis, allergic rhinitis, Japanese pollinosis, allergic conjunctivitis, urticaria, and food allergy, as evidenced by the description of the findings by the allergologists.

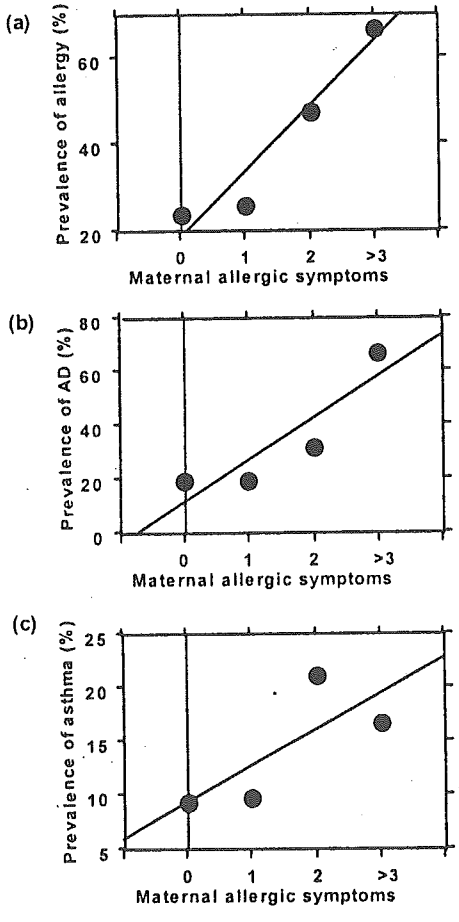

Fig. 3 The prevalence of infantile allergy and the number of allergic symptoms in the mothers during pregnancy. The rate of development of total childhood allergy including atopic dermatitis and asthma (a) and that of atopic dermatitis in isolation (b) but not that of asthma in isolation (c) correlated with the frequency of the maternal allergic signs. AD, atopic dermatitis.

Association of maternal allergic symptoms after delivery with the allergy in their children

In addition to maternal allergies during pregnancy, maternal allergic diseases during the child-bearing period appeared to lead to a markedly increased prevalence of allergy in their progeny (p < 0.01) (Fig. 4). Similarly, the numbers of maternal allergic symptoms after childbirth significantly paralleled the prevalence of the sum of allergic diseases (both atopic dermatitis and asthma) (rs = 0.985658, p < 0.01), atopic dermatitis in isolation (rs = 1, p < 0.01), and asthma in isolation (rs = 1, p < 0.01) (Fig. 5).

Relationship of allergic diseases in the infants to those of their family members

The childhood incidence of allergic dis-

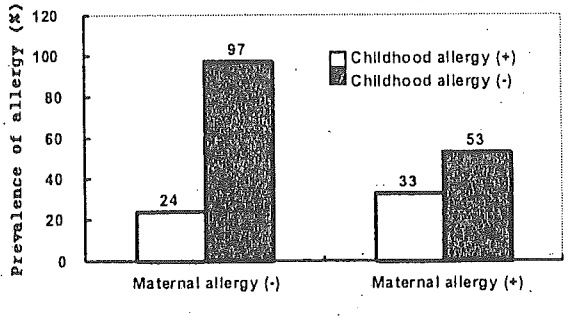

Fig. 4 The influence of maternal allergy during the childhood-bearing period on the prevalence of allergy in their progeny. Open columns and closed columns indicate children with and without allergy, respectively.

eases ($rs = 1$, $p < 0.01$), either in individuals with atopic dermatitis plus asthma, or atopic dermatitis in isolation ($rs = 1$, $p < 0.01$), or asthma in isolation ($rs = 1$, $p < 0.01$) was strongly linked with the numbers of the allergic individuals in their families (Fig. 6).

Influence of infectious diseases early in life on the development of allergy in the children

Based on the results of the questionnaire regarding the diagnosis and the time of the childhood respiratory infectious diseases, no childhood infectious respiratory diseases in isolation had any influence on the prevalence of allergic diseases (data not shown).

Association between the nutrition and allergic signs in the infants

The feeding of the children, which was either breast or cows milk, irrespective of the duration, did not affect the development of allergy (data not shown).

Prevalence in the onset of allergic diseases in regard to vaccinations

The prevalence of childhood allergy did not differ among the groups that received DPT, BCG

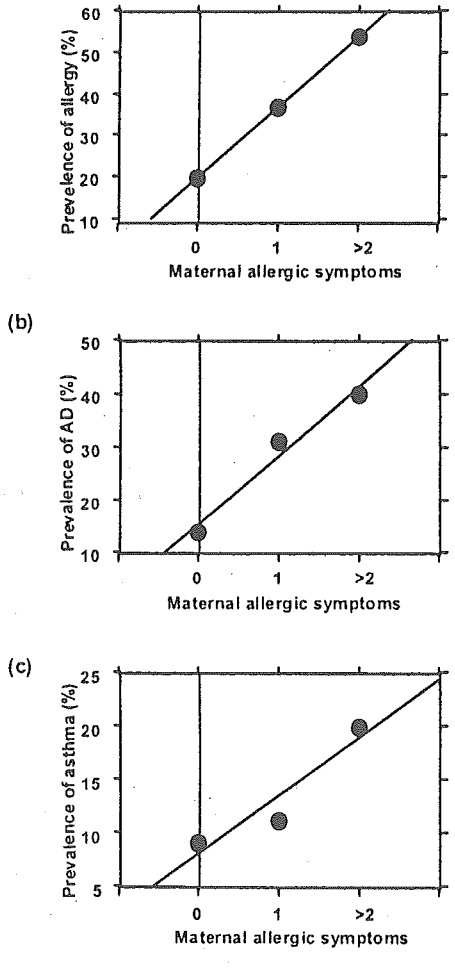

Fig. 5 Correlations of the rate of development of infantile allergy with the number of the allergic symptoms in their mothers. The prevalence of atopic dermatitis plus asthma (a) and that of atopic dermatitis in isolation (b), but not that of asthma in isolation (c) paralleled the maternal allergy. AD, atopic dermatitis.

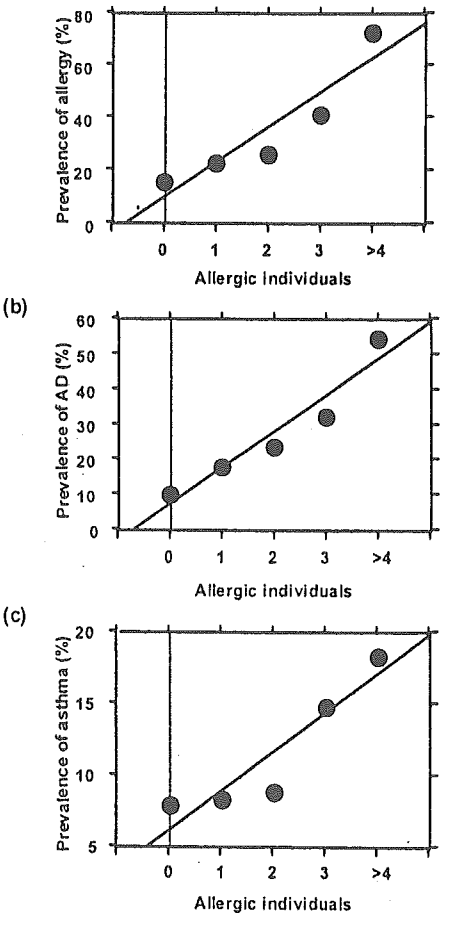

Fig. 6 Significant correlations between childhood allergy and a family history of allergy. The development of atopic dermatitis plus asthma (a), that of atopic dermatitis in isolation (b), and that of asthma in isolation (c) was influenced by the number of patients experiencing allergy in the family. AD, atopic dermatitis.

vaccination, polio, measles, rubella, varicella, mumps, or influenza virus vaccinations (data not shown). The effect of immunizations for diphtheria and tetanus (DT) or for the Japanese encephalitis virus could not be assessed due to the small numbers of such cases.

Influence of indoor pets on the allergic symptoms

The presence or absence of pets, either in the prenatal or the postnatal period, and the duration of their exposure, especially cats and dogs, had no influence on the allergic symptoms in the children (data not shown).

Association of environmental tobacco smoke and allergy in the children

The homes of smokers or nonsmokers did not affect allergy in their infants. And the development of childhood allergy was not changed by the time of exposure and the extent of any smoking (data not shown).

DISCUSSION

Previous studies have depicted several factors that might be related to the development of childhood allergy, such as nutrition, environments with the presence of mites, cats and tobacco, different lifestyles including the occupation and the economic status, any genetic predisposition and infantile infections. These may represent potential determinants.[7,8] In addition to the above-mentioned factors in postnatal life, prenatal intrauterine sensitization to the allergen might be responsible for the development of allergy in their offspring, as allergen-specific T lymphocytes have been reported to be present as early as 22 weeks of gestation.[2]

The present study was undertaken to analyze the possible risk factors for triggering allergy, such as pre- and post-natal maternal symptoms, nutrition, vaccination, and any exposure to animals and tobacco smoke.

Our data showing that the frequency of maternal infections during pregnancy, including diarrhea, fever, rhinorrhea, cough, pharyngitis, bronchitis, and pneumonia strongly correlated with the prevalence of allergy in their offspring, suggest that immunological alterations of the infants could be caused by maternal infections.

Although we have no definite evidence for a link between maternal pharyngitis and atopic dermatitis in their infants, immunological alterations during pregnancy could modify fetal immunity as was reported elsewhere:[9] maternal immunization to dust mites carried out prior to conception inhibited the type I hypersensitivity response of the offspring in an antigen-specific way. Thus, the study and our data suggest that several maternal factors which cause immunological changes during pregnancy also trigger the development of allergy in the offspring. In this scenario, immune cells residing in the mother's pharynx in inflammation were activated by epithelial cells which highly expressed thymic stromal lymphopoietin (TSLP),[10] and subsequently secreted cytokines and mediators. These, in turn, stimulated fetal lymphocytes and enabled these to migrate to the skin of the children. In fact, skin-homing chemokines (ligands) and their receptors include CCL17/TARC (thymus- and activation-regulated chemokine) and CCL22/MDC (monocyte-derived chemokine) and their receptor CCR4, CCL27/CTAC (chemotactic responsiveness to cutaneous T-cell-attracting chemokine) and its receptor CCR10, and E-selectin and its receptor cutaneous lymphocyte-associated antigen (CLA),[11] either of which may be affected by the cytokines and mediators released from the mother's pharynx.

A link between the family history of allergy and the development of allergy in the progeny reflects the active role of both genetic and environmental factors, as reported elsewhere.

In our study, infantile infections were not associated with the development of allergy. Our investigation should, however, be extended to cover the use of antibiotics, because they may increase the incidence of allergic diseases as reported previously.[12]

The present study did not find any link between the feeding of the infants and the development of allergy. Conflicting results have been obtained with regard to the influence of breast-feeding on infantile allergy: breast-feeding may lower the incidence of allergy in children,[13] whereas it may also be a risk factor for the development of allergy.[14] Exten-

sive study in the near future should define the role of breast-feeding on allergic childhood diseases.

In the context of immune dysregulation and allergy, several vaccinations have been linked to the development of allergic diseases in infancy. Particularly noteworthy is the influence of BCG and DPT: BCG has reportedly attenuated the incidence of allergy,[15,16] while the DPT vaccination may promote atopic disorders.[16] The results regarding BCG and DPT in our study disagreed with that. This discrepancy may have resulted from the difference in ages of the individuals studied.

Neither exposure to pet nor environmental tobacco smoke was identified as a risk factor for the development of allergy in this study. The results of the present investigation differ from those showing a positive link between exposure to cats and environmental smoke and childhood allergy.[7] Our study includes only children aged under two years old, inevitably excluding those who would otherwise develop allergic diseases after this age.

In combination, the pre- and post-natal factors that may affect the immune system of the fetus and infants were clearly shown to promote or inhibit the development of allergy respectively. We hope the avoidance of the aforementioned risk factors may prevent the development of childhood allergy.

ACKNOWLEDGMENT

This study was partly supported by Research on Allergic Diseases and Immunology of Health and Labour Sciences Research Grants in Japan.

REFERENCES

1. Holt PG. Parasites, atopy, and the hygiene hypothesis: resolution of a paradox? Lancet 2000; 356: 1699-1701.
2. Warner JA, Jones AC, Miles EA, Colwell BM, Warner JO. Maternofetal interaction and allergy. Allergy 1996; 51: 447-51.
3. Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). Eur Respir J 1998; 12: 315-5.
4. Williams HC, Burney PG, Pembroke AC, Hay RJ. The U.K. Working Party's diagnostic criteria for atopic dermatitis. III. Independent hospital validation. Br J Dermatol 1994; 131: 406-16.
5. Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol 1980; 92: 44-7.
6. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, November 1986. Am Rev Respir Dis 1987; 136: 225-44.
7. Wahn U, von Mutius E. Childhood risk factors for atopy and the importance of early intervention. J. Allergy Clin. Immunol. 2001; 107: 567-74.
8. O'Connell EJ. Pediatric allergy: a brief review of risk factors associated with developing allergic disease in childhood. Ann Allergy Asthma Immunol 2003; 90(6 Suppl 3): 53-8.
9. Victor JR, Jr., Fusaro AE, Duarte AJ, Sato MN. Preconception maternal immunization to dust mite inhibits the type I hypersensitivity response of offspring. J Allergy Clin Immunol 2003; 111: 269-77.
10. Soumelis V, Reche PA, Kanzler H, *et al.* Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nat Immunol 2002; 3: 673-80.
11. Soler D, Humphreys TL, Spinola SM, Campbell JJ. CCR4 *versus* CCR10 in human cutaneous TH lymphocyte trafficking. Blood 2003; 101: 1677-82.
12. McKeever TM, Lewis SA, Smith C, *et al.* Early exposure to infections and antibiotics and the incidence of allergic disease: a birth cohort study with the West Midlands General Practice Research Database. J Allergy Clin Immunol 2002; 109: 43-50.
13. Chulada PC, Arbes SJ, Jr., Dunson D, Zeldin DC. Breastfeeding and the prevalence of asthma and wheeze in children: analyses from the third national health and nutrition examination survey, 1988-1994. J Allergy Clin Immunol 2003; 111: 328-36.
14. Sears MR, Greene JM, Willan AR, *et al.* Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: a longitudinal study. Lancet 2002; 360: 901-7.
15. Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. Science 1997; 275:77-9.
16. Yoneyama H, Suzuki M, Fujii K, Odajima Y. The effect of DPT and BCG vaccinations on atopic disorders. Arerugi 2000; 49: 585-92.

Original Paper

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2005;138:51–58
DOI: 10.1159/000087357

Received: August 30, 2004
Accepted after revision: March 29, 2005
Published online: August 9, 2005

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.

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-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–2438/05/1381–0051$22.00/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Kenichi Tokuyama, MD
Department of Pediatrics, Gunma University Graduate School of Medicine
3-39-22 Showa-machi, Maebashi, Gunma 371-8511 (Japan)
Tel. +81 27 220 8205, Fax +81 27 220 8215
E-Mail tokuyama@showa.gunma-u.ac.jp

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 ($AlOH_3$) 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 cytospin 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 H_2O 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:

Mucus area (%) = (mucus area/total epithelial area) × 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* $NaHCO_3$. 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 H_2SO_4, 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

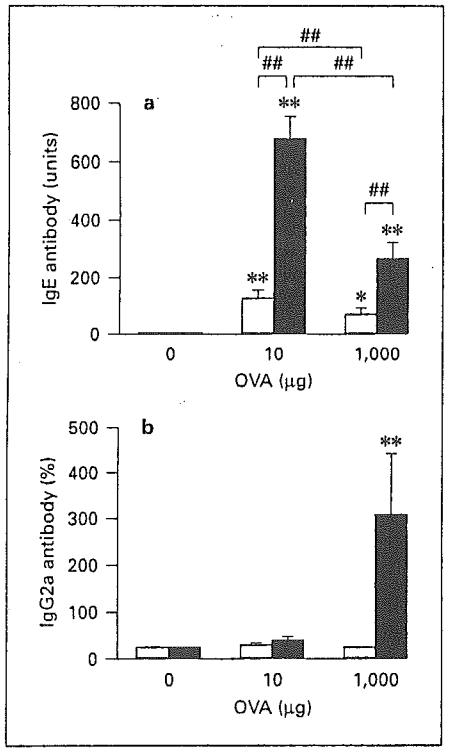

Fig. 1. OVA-specific IgE (**a**) and IgG2a levels (**b**). The open and closed columns indicate the values of juvenile and adult animals, respectively. Values are expressed as mean ± SEM. * $p < 0.05$ and ** $p < 0.01$ vs. control animals of the corresponding ages, respectively. ## $p < 0.01$ between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

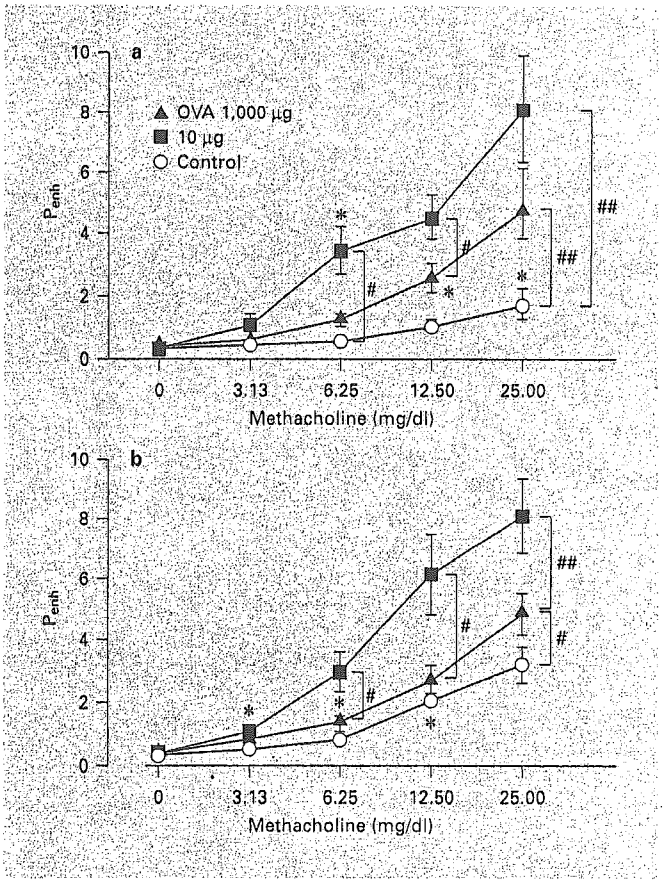

Fig. 2. Bronchial responsiveness to aerosolized methacholine in juvenile (**a**) and adult mice (**b**). Dose-response curves in animals given 10 and 1,000 μg OVA and vehicle (control) are shown. Values are expressed as mean ± SEM. # $p < 0.05$ and ## $p < 0.01$ between groups. * Indicates PC200 for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

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 ± 0.0	121.6 ± 62.1*	18.8 ± 6.8*
Adult	8.6 ± 5.6	88.5 ± 38.8*	14.6 ± 8.3
IFN-γ, ng/ml BALF			
Juvenile	75.5 ± 32.5	50.8 ± 33.1	86.2 ± 26.2
Adult	120.6 ± 29.8	13.5 ± 13.5*	77.7 ± 36.0

Values are expressed as mean ± SEM.
* $p < 0.05$ vs. the corresponding animals given vehicle for OVA (control) (n = 4–6 mice for each group.)