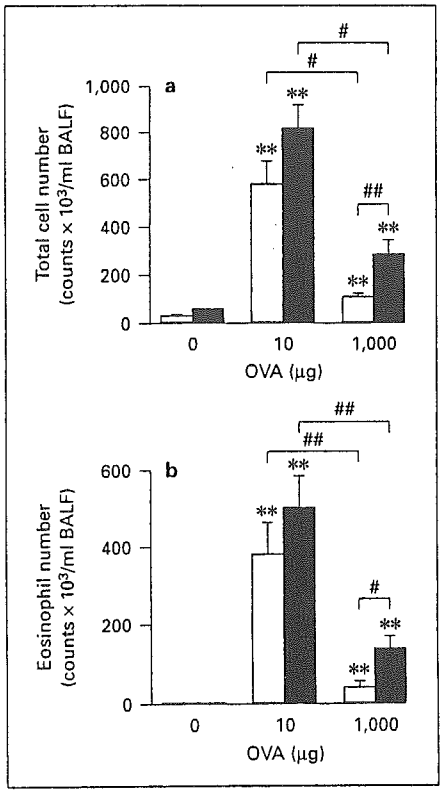

Fig. 3. The numbers of total cells (**a**) and eosinophils (**b**) in BALF. The open and closed columns indicate the values of juvenile and adult animals, respectively. Values are expressed as mean ± SEM. ** $p < 0.01$ vs. control animals with the corresponding age. # $p < 0.05$ and ## $p < 0.01$ between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

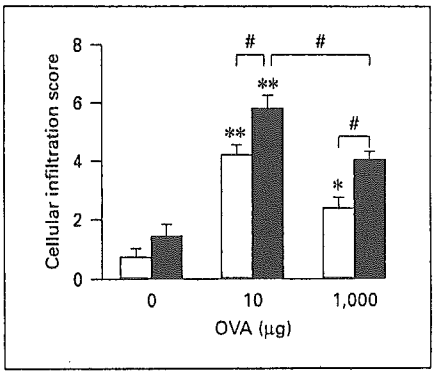

Fig. 4. Cellular infiltration score. The open and closed columns indicate the values of juvenile and adult animals, respectively. Values are expressed as mean ± SEM. * $p < 0.05$ and ** $p < 0.01$ vs. control animals with corresponding ages, respectively. # $p < 0.05$ between groups (n = 8–10 mice for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

lation was observed between animals given 10 μg and 1,000 μg 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 μg 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 μg than in those given 1,000 μg OVA (fig. 3a, b, respectively). In juvenile animals given 1,000 μg OVA, the increases in the number of total cells and eosinophils were scarce compared with the other 3 groups of animals given OVA.

Histopathological Findings of the Lung

In both adult and juvenile mice, the cellular infiltration score was more prominent in animals given 10 μg 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 μg than in those given 1,000 μg 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.

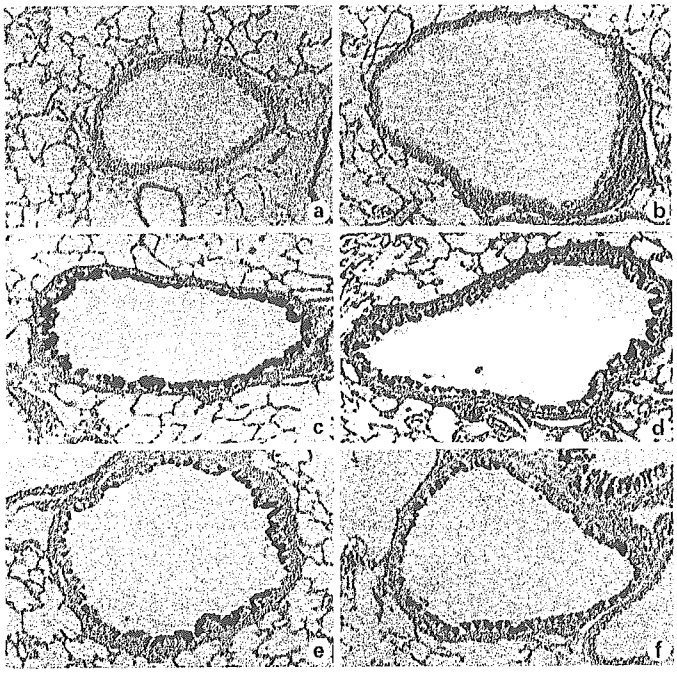

Fig. 5. Representative photomicrographs of 6-μm sections of mouse lung (AB/PAS staining). The sections of control animals **a**: juvenile; **b**: adult animals) and those of animals sensitized and challenged with 10 (**c**: juvenile; **d**: adult animals) and 1,000 μg of OVA (**e**: juvenile; **f**: 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 × 100).

Table 2. The mucus cell score and the mucus area

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

Values are expressed as mean ± 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, μg OVA induced elevated IL-4 and decreased IFN-γ levels in BALF in both age groups, suggesting that 10 μg OVA induced Th2-dominant conditions whereas 1,000 μg 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 μg than in those sensitized with 1,000 μg 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 μg 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 μg 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 μg induced a Th1-biased environment compared to that with 10 μg OVA in adult mice. In contrast to adult animals, even 1,000 μg OVA still induced a Th2-biased immune environment in juvenile animals, although the degree was less than in those given 10 μg 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 μg 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 μg 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.

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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, Agustí 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, Cory 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: Maturational 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.

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Effect of Disodium Cromoglycate on Airway Mucus Secretion during Antigen-Induced Late Asthmatic Responses in a Murine Model of Asthma

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Key Words
Asthma · Airway · Disodium cromoglycate · Mucin

Abstract
Background: Disodium cromoglycate (DSCG) is known to inhibit both immediate and late asthmatic responses (IAR and LAR). However, its effect on mucus hypersecretion is unknown. Using a murine model of asthma, we aimed to determine whether mucus secretion increased during IAR and LAR. We also studied the potency of DSCG in inhibiting mucus secretion and on airway eosinophilia. ***Methods:*** Mice were subjected to initial intraperitoneal sensitization and airway challenge to ovalbumin (OVA) and then provoked by additional exposure to OVA. Some mice were pretreated with aerosolized DSCG (20 mg/ml) 1 h before the provocation with OVA. After serial measurements of enhanced pause (Penh), an indicator of airflow obstruction, serum samples and bronchoalveolar lavage fluids (BALF) were collected. Then, the lungs were excised and a morphometric analysis for mucus hypersecretion was performed. ***Results:*** A biphasic increase in Penh (IAR and LAR) was observed in sensitized animals after provocation with OVA. Airway eosinophilia was observed during both responses. Intraluminal mucus significantly increased during LAR, but not during IAR. DSCG significantly attenuated both IAR and LAR, and significantly inhibited the increase in intraluminal mucus during LAR, but had no effect on eosinophilia in BALF. ***Conclusion:*** Our results suggest that airway hypersecretion may be involved as a component of airflow obstruction during LAR, and that this is unlikely during IAR. DSCG may be effective in reducing excessive airway mucus caused by exposure to allergens.

Introduction

Mucus hypersecretion from mucus-secreting cells is an important component of airflow obstruction seen in airway diseases such as asthma. In a subgroup of asthma, allergen provocation leads to biphasic bronchoconstrictive responses, i.e. immediate asthmatic response (IAR), peaking at 15–30 min and returning to baseline within 1–2 h, and late asthmatic response (LAR), commencing 3–5 h after provocation and peaking at 6–12 h [1, 2]. The mechanisms and the airway cells involved in IAR and LAR may differ because the efficacy of each antiasthma drug differs against each of these responses. It is likely that IAR mainly depends on airway smooth muscle contraction in view of its rapid onset and prompt return to the baseline level in lung function. By contrast, mucus hypersecretion may, at least in part, contribute to the development of LAR considering its slow and persistent airway obstruction.

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The mechanism of airway mucus secretion in respiratory diseases such as asthma has been studied mainly using morphometric analysis in humans and in experimental animals [3–5]. We showed that neurogenic inflammation induced airway goblet cell discharge in guinea pigs by developing a method in which the mucus score was measured as an index of secretion [3]. In humans, Aikawa et al. [6] quantified intraluminal mucus and demonstrated that mucus hypersecretion could be an important component of airway obstruction in chronic bronchitis. In guinea pigs, Hashimoto et al. [5] showed that pretreatment with diesel exhaust induced the formation of mucus plugs in the airway lumen 15 min after allergen challenge in sensitized animals. This finding indicates that mucus hypersecretion may contribute to the airway responses caused by allergen exposure in sensitized individuals.

Disodium cromoglycate (DSCG) is a widely used antiasthma drug, especially for younger children with asthma. DSCG has been shown to inhibit both IAR and LAR in humans [7] and in experimental animals [8]. The main mechanism of DSCG in inhibiting antiallergic action is thought to be via the mast cell stabilizing effect, although other airway effects, such as sensory nerve inactivation [9] and tachykinin antagonist activity [10], have been proposed.

In the present study, we wanted to clarify whether mucus secretion increased during airway obstruction caused by allergen exposure. For this purpose, we employed a murine model of asthma [8] in which the animals showed both IAR and LAR after provocation with antigen. We quantified intraluminal mucus, as well as airway eosinophilia, during IAR and LAR, and evaluated the inhibitory effects of DSCG against these responses.

Materials and Methods

Sensitization, Airway Challenge and Provocation with Ovalbumin (OVA)

All of the experimental animals in this study were used in accordance with a protocol approved by the Institutional Animal Care and Use Committee.

Principally, the method reported by Cieslewicz et al. [8] was employed for the sensitization and airway challenge of the animals. In brief, BALB/C mice, 10–12 weeks of age, were sensitized on days 1 and 14 by intraperitoneal injection of 20 μg OVA (grade V; Sigma, St. Louis, Mo., USA) emulsified in 2.25 mg aluminum hydroxide (Wako, Tokyo, Japan) in a total volume of 100 μl. On days 28, 29 and 30, the mice were challenged daily with OVA (1% in saline) for 20 min via the airways using an ultrasonic nebulizer (NE-U12, Omron, Tokyo, Japan) in a 4.5-liter inhalation box. On day 32, the mice were provoked with OVA (5% in saline) or saline for 20 min. Following this, airway responsiveness was determined at 5, 15, 30 and 60 min, and then at intervals of 30 min for the next 6 h using whole-body barometric plethysmography. In another group of animals, airway responsiveness was measured for 12 h after the provocation to observe the time course of LAR.

Study Protocol

In a pilot study (study I) in which airway responsiveness was observed for 12 h after provocation, mice were sensitized, challenged with OVA and provoked with either OVA (n = 6) or saline (n = 5) to confirm that both IAR and LAR appeared in the present model.

In a second study (study II), the degree of mucus secretion and the number of eosinophils in BALF during IAR or LAR were measured and the inhibitory effects of DSCG on these responses were examined. All of the animals were sensitized, challenged with OVA and then treated with either DSCG (20 mg/ml diluted with saline) or the vehicle (saline) 1 h before provocation. After provocation with either OVA or the vehicle (saline), the animals were killed at 15 min or at 6 h following the measurement of airway responsiveness. Thus, there were six groups of animals: two groups pretreated with the vehicle for DSCG 1 h before provocation with OVA and killed either 15 min (IAR) or 6 h (LAR) after provocation (n = 10 and n = 13, respectively). Ten of the animals treated with DSCG before provocation with OVA were killed at 15 min and the remaining 16 animals 6 h after provocation. Control animals were treated with the vehicle for DSCG prior to provocation with saline and were then killed at the same time points (n = 10 and n = 10, respectively).

Determination of Airway Responsiveness

The time course of airway responsiveness after the antigen provocation was studied on unstrained, conscious mice as described previously [11, 12]. 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 (Penh), was calculated. It has recently been shown that Penh does not always correlate with airway resistance [13], but we used Penh as the parameter because this was suitable for the continuous measurement of the time course of airflow obstruction.

Bronchoalveolar Lavage (BAL) Procedure

After the measurement of airway responsiveness, the animals were killed with an overdose of pentobarbital (50 mg/animal i.p.) in order to obtain serum samples. After taking the samples, a 18-gauge cannula was introduced into the proximal portion of the trachea, and the lungs were lavaged with 0.4 ml of phosphate-buffered saline 3 times. The BAL fluids were centrifuged at 800 rpm for 5 min. Cell pellets were resuspended in 0.3 ml of RPMI-1640 medium. Total cell counts were performed with a hemocytometer, and differential cell counts were performed on cytospin preparations stained with Diff-Quick (Kokusai-Shiyaku, Tokyo, Japan). A blinded observer counted a minimum of 200 cells for each sample.

Tissue Preparation

After the BAL procedure was completed, the lungs were inflated to a pressure of 25 cm H_2O. The trachea was clamped until fixation was complete. Tissue specimens were cut into 6-μm-thick sections at mid-sagittal slices in the mid-sagittal plane, embedded in paraffin and stained with Alcian blue and periodic acid-Schiff (AB/PAS). Slides were coded and graded in a blind fashion.

Quantification of Intraluminal Mucus

The mucosubstance area in the airway lumen was quantified by evaluating mucus areas coated in mucus morphometrically. In brief, the stained slides were examined with a light microscope (IX 70; Olympus, Tokyo, Japan) connected to a color chilled CCD camera system (M 3204C; Olympus) and to a color digital graphic tablet (Intuos GD-0608-R; Wacom, Saitama, Japan). Images of all membranous airways in the slide were recorded. Analysis was performed on a personal computer (PC 300 GL, IBM, New York, N.Y., USA) with images displayed on the color monitor using graphic analysis software (Win ROOF version 3.41; Miitani, Fukui, Japan). The length of the inner perimeter (Pi) of each airway lumen was measured and the luminal size of each airway calculated, assuming a circular airway [4, 6], in accordance with the formula: luminal size (mm^2) = $Pi^2/4\pi$.

All peripheral airways that were cut vertically in section profile with inner perimeters ranging from 0.79 to 3.5 mm were evaluated for morphometrical analysis of intraluminal mucus. Preliminary studies showed that there was no epithelial goblet cell metaplasia in unsensitized animals or in airways smaller than 0.79 mm even in sensitized animals. Therefore, airway lumens with Pi <0.79 mm were not measured.

All areas of intraluminal mucus in each slide (areas stained purple by AB/PAS) were measured and added together. The mucus areas in each animal were then defined using the formula:

Mucus area (mm^2) = total area of intraluminal mucus/number of airway lumens examined.

OVA-Specific IgE Antibody Determination

OVA-specific IgE levels were determined by ELISA, as described previously [14]. In brief, 96-well microtiter plates (Dynex, Tokyo, Japan) were coated with 200 μg/ml of OVA (grade V; Sigma) diluted in 0.1 *M* $NaHCO_3$ and incubated for 2 h at 37°C. After washing and blocking with PBS-bovine serum albumin for 2 h at 37°C, serially diluted 100-μl serum samples were added and incubated for 2 h at 37°C. After washing, 100 μl of 1:800-diluted rat anti-mouse IgE monoclonal antibody (Biosource International, Camarillo, Calif., USA) were added and incubated for 2 h at 37°C. Then, the reaction chromogen was generated with FAST (Sigma), and stopped with H_2SO_4. Plates were read in a multiplate reader at 490 and 620 nm. Serum pooled from OVA-sensitized and airway-challenged adult mice was used as a positive control. The OVA-specific IgE titer was determined to be the reciprocal of the highest dilution giving a positive value (arbitrary unit: AU).

Statistical Analysis

All data were expressed as means ± SEM. Non-parametric analysis of variance (the Kruskal-Wallis test for unmatched pairs) was used to determine the significance of variance between groups. If a significant variance was found, 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, and a Bonferroni correction was performed when required. As for the level of significance of Penh during IAR and LAR, the area under the curve during corresponding time points was compared. Statistical analysis was performed utilizing Statview version 4.5 (Avacus Concepts, Berkeley, Calif., USA).

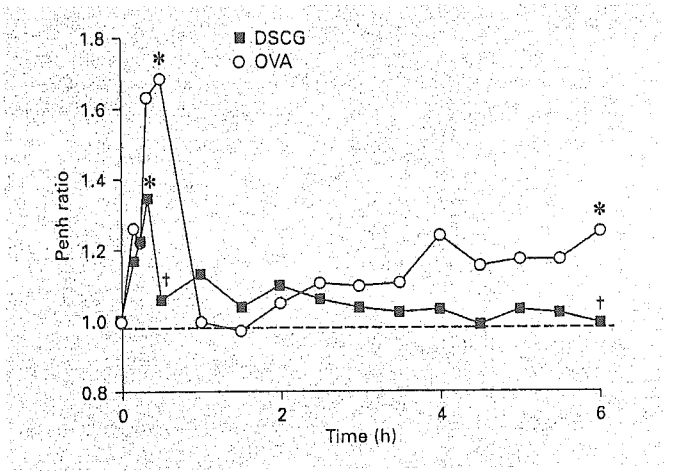


Fig. 1. The effect of DSCG on the increase in Penh up to 6 h after the provocation. Penh was expressed as the ratio compared to the baseline level before the provocation. The values are means at each time point. DSCG significantly attenuated the increase in Penh caused by OVA in both phases († $p < 0.05$). * $p < 0.05$ compared to control animals given vehicle for OVA.

Results

Airway Responsiveness after Allergen Exposure

In study I, provocation with 5% OVA induced both IAR and LAR. An increase in Penh was observed as early as 5 min after provocation, peaking at 15 min, returning to initial values by 90 min. The Penh at IAR was statistically significant compared to that in mice sensitized and challenged with OVA and provoked with saline ($p < 0.05$). In mice provoked with antigen, a second increase in Penh (LAR) started at around 2–3 h, and peaked at 8.25 h on average. Then, the value of Penh declined in all animals. In 3 of these animals, the value declined to the baseline level by 12 h after the provocation. However, it was still higher than the baseline level in another 3 animals at the time point. As in IAR, Penh at LAR was significantly greater than that in mice sensitized and challenged with OVA and provoked with saline ($p < 0.05$).

In study II, provocation with OVA induced IAR and LAR. The values in Penh at both IAR and LAR were significantly higher than those in animals treated with the vehicle for OVA ($p < 0.05$ at both IAR and LAR). DSCG significantly attenuated both IAR and LAR ($p < 0.05$ at both responses), whereas Penh during IAR (not during LAR) in DSCG-treated animals was significantly higher than that in control animals treated with the vehicle ($p < 0.05$; fig. 1).

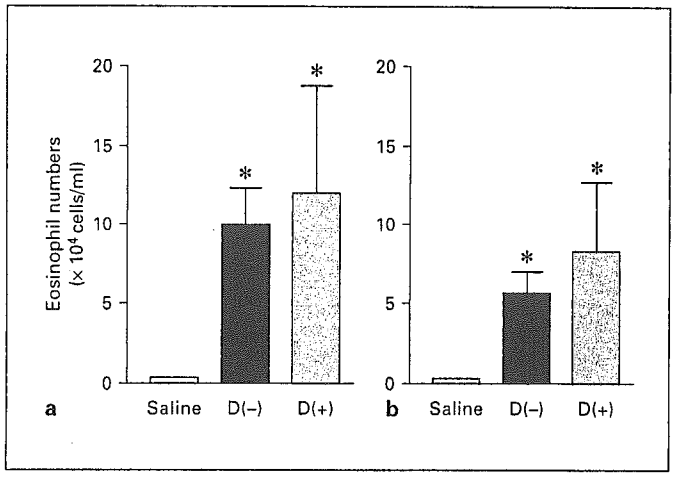


Fig. 2. The number of eosinophils in BALF during IAR (**a**) and LAR (**b**). Values are means ± SEM. D(−) and D(+) indicate OVA-challenged animals without or with pretreatment of DSCG, respectively. Saline = Control animals given vehicle for OVA and sacrificed at the corresponding time points. A marked elevation in eosinophil counts was observed both at IAR (**a**) and at LAR (**b**). DSCG did not affect the number of eosinophils both at IAR and LAR. * $p < 0.05$ compared to control animals given vehicle for OVA.

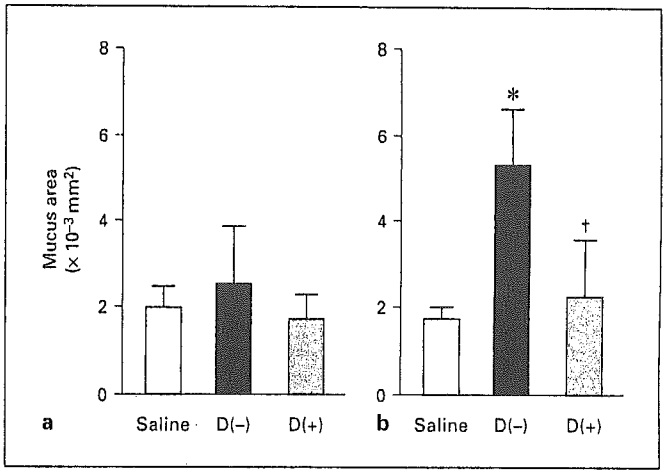


Fig. 3. The mucus area during IAR (**a**) and LAR (**b**). Values are means ± SEM. D(−) and D(+) indicate OVA-challenged animals without or with the pretreatment of DSCG, respectively. Saline = Control animals given vehicle for OVA and sacrificed at the corresponding time points. Mucus areas increased during LAR in OVA-challenged animals. DSCG significantly inhibited the increase during LAR. * $p < 0.05$ compared to control animals at the corresponding time points. † $p < 0.05$ compared to animals given OVA without pretreatment of DSCG.

Table 1. Mean value of the luminal size in each group

Group	n	Luminal size $\times 10^{-3}$ mm^2
IAR		
Saline	10	108.3 ± 24.0
D(−)	10	135.0 ± 22.8
D(+)	10	112.9 ± 22.8
LAR		
Saline	10	102.4 ± 21.8
D(−)	13	124.5 ± 21.0
D(+)	16	102.5 ± 5.6

Values are means ± SEM. D(−) and D(+) indicate OVA-challenged animals without or with pretreatment of DSCG, respectively. Saline means control animals given vehicle for OVA and sacrificed at the corresponding time points. No significant difference in the size of airway lumina was observed among the six groups.

Airway Eosinophilia

In animals not pretreated with DSCG, a marked elevation in the number of eosinophils in the BALF was observed during both IAR and LAR (9.87 ± 2.71 × 10^4 and 5.59 ± 1.28 × 10^4 cells/ml, respectively). These numbers were significantly higher than those in control animals killed at corresponding time points (0.018 ± 0.011 × 10^4 and 0.015 ± 0.015 × 10^4 cells/ml, respectively; $p < 0.05$ and $p < 0.05$, respectively). DSCG had no effect on the number of eosinophils during IAR or LAR (fig. 2).

Intraluminal Mucus

The luminal size was not significantly different among the six groups (table 1). Morphometric analysis revealed that the mucus area in the airway lumen (× 10^{-3} mm^2) was significantly greater at LAR (5.28 ± 1.07, $p < 0.05$) but not at IAR (2.49 ± 1.21) in animals provoked with OVA than in those given the vehicle for OVA (1.93 ± 0.58 and 1.70 ± 0.51 at the time points of IAR and LAR, respectively). In DSCG-treated animals, the mucus areas at the time points of IAR and LAR were comparable with those in control animals (1.66 ± 0.74 and 2.21 ± 1.27, respectively). DSCG significantly inhibited the increase in the mucus areas at LAR ($p < 0.05$, fig. 3). A photograph typical of each group is shown in figure 4. Areas quantified for Pi measurement are marked in the figure.

Analysis of OVA-Specific IgE

The serum level of OVA-specific IgE was markedly elevated in all groups of animals subjected to sensitization and challenge (range: 80–20,480 AU). In the three groups of animals examined at the time point of IAR, the mean values of the IgE titer (AU) were 4,608 ± 512, 10,752 ± 3,999 and 15,360 ± 3,238 in animals without provocation, and those with provocation of OVA without and with pretreatment of DSCG, respectively. In the three groups of animals examined at the time point of LAR, the mean values of the IgE titer (AU) were 12,288 ± 2,048, 8,192 ± 1,254 and 8,288 ± 4,977 in animals without provocation, and those with provocation of OVA without and with pretreatment of DSCG, respectively. No significant difference in the IgE titer was found among the six groups.

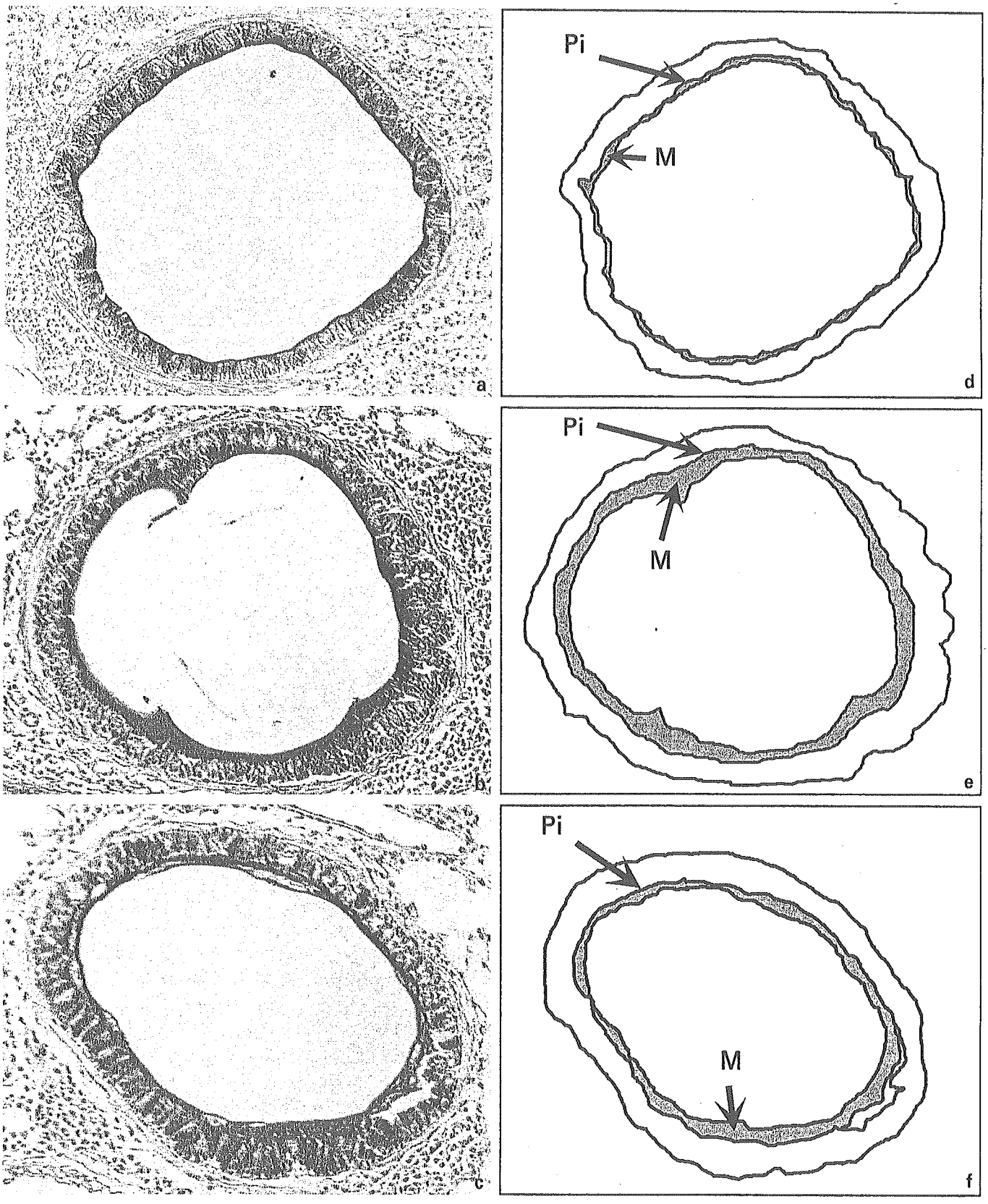
Discussion

In a murine model of asthma in which both IAR and LAR were observed after antigen provocation, we demonstrated that the quantity of mucus in the airway lumen significantly increased during LAR, but not during IAR. DSCG significantly attenuated the amount of intraluminal mucus in LAR, but did not affect airway eosinophilia during IAR or LAR. It is difficult to directly evaluate the amount of degranulated intraluminal mucus. Thus, we performed a morphometric analysis to quantify the amount of mucus during antigen-induced airway responses. In the present study, we quantified the intraluminal mucus after performing the BAL procedure. It has been shown that the recovery rates of substances in intraluminal fluids differ depending upon several conditions, e.g. viscosity [15]. Thus, it is likely that mucus components that are difficult to remove from the airways by BAL may be responsible for the difference in the amount of intraluminal mucus in the present study. Measuring the content of mucins in the BALF would be useful to determine the degree of secretion, but we did not do so in the present study.

In humans, even mild asthma is associated with goblet cell hyperplasia and increased stored mucin in the airway epithelium, whereas moderate asthma is associated with increased stored mucin and secreted mucin [16]. These findings suggest that acute degranulation from hyperplastic goblet cells may be the cause of asthma exacerbations in mild and moderate asthma, and that chronic degranulation from goblet cells may contribute to chronic airway narrowing in moderate asthma. In rodents, allergic sensitization with OVA has been shown to cause goblet cell metaplasia [17], and the Th2 cytokines IL-4 and IL-13 have been implicated in this process [18]. In the present study, we demonstrated that mucus-secreting cells in sensitized mice degranulated greater amounts of intraluminal mucus during LAR, but not during IAR, indicating that airway mucus hypersecretion may be a component of airflow obstruction during LAR, but not during IAR. However, we do not know the exact role of the excess mucus during LAR. It has been shown that other events such as increased plasma exudation and eosinophilic inflammation occur during LAR. Thus, a combination of these events with mucus hypersecretion may be responsible for long-lasting airflow obstruction, a characteristic feature of LAR. In contrast to our results, several studies in asthmatic subjects have shown that there might be no degranulation of goblet cells after allergen challenge [19]. We can only surmise that this difference between results may be due to a difference in species: further research is required to clarify this matter.

In the present study, we could not clarify the mechanism of mucus hypersecretion during LAR. Various kinds of secretagogues causing the degranulation of airway mucus have been proposed and include environmental stimuli such as smoke and ammonia, local release of neuropeptides or activation of cholinergic reflex pathways, and inflammatory mediators such as neutrophil elastase, mast cell chymase, leukotrienes, histamine and non-protease neutrophil products [20]. Thus, one or more factors among them may contribute to the degranulation after antigen exposure. In the present study, DSCG had no effect on the number of eosinophils in BALF, during IAR or during LAR, confirming previous reports in guinea pigs [21] and in mice [22], whereas it significantly decreased the amounts of intraluminal mucus during LAR. Our results may indicate that the activation of eosinophils is not required for the degranulation process of airway mucus after antigen exposure. In experimental animals, it has been shown that eosinophils are not required for allergen-induced goblet cell metaplasia. Cohn et al. [23] showed that airway mucus production did not decrease even when airway eosinophils are progressively eliminated from the lung. Based on this finding, they speculated that the association between airway eosinophilia and increased mucus production seen in mice merely reflects the association between eosinophils and activated Th2 cells secreting IL-5. Further studies are required to clarify the role of eosinophils in mucus degranulation.

A single application of DSCG is shown to be sufficient to inhibit both IAR and LAR, not only in humans [7] but


a
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also in experimental animals [8]. Our present study confirms previous observations. Also, we showed that DSCG may be potent in inhibiting intraluminal degranulation of airway mucus during LAR. As far as we know, there has been no report that DSCG has any effect on mucus production or mucus gene expression. We were not able to clarify the exact mechanism by which DSCG inhibited mucus hypersecretion. Because DSCG is known to have a mast cell stabilizing effect, it is likely that it inhibits mucus secretion secondary to inhibiting mast cell function. Mast cells are known to induce allergic airway inflammation via the release of various cytokines and chemokines such as IL-4, IL-13 and eotaxin; inflammatory mediators such as histamine, cysteinyl leukotrienes and platelet-activating factor; and free radicals such as the superoxide anion. It has been suggested that several of these substances may be the secretagogues causing degranulation of airway mucus. Thus, it is reasonable to

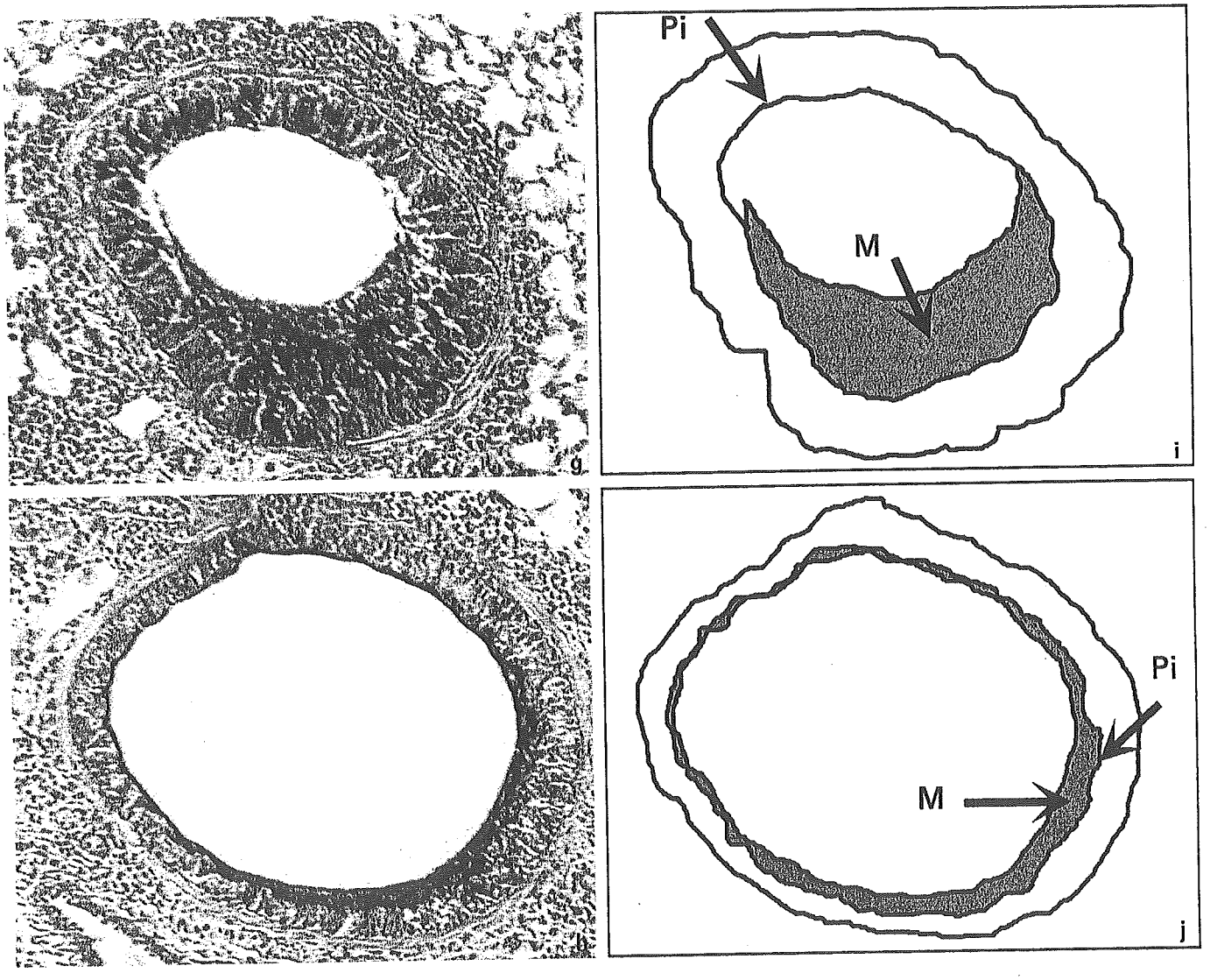

Fig. 4. Representative photomicrographs of 6-μm sections of mice lung (AB/PAS staining). The sections of the control animal sacrificed 15 min after the provocation with vehicle for OVA (**a**), those at IAR without or with DSCG (**b**, **c**, respectively), and those at LAR without or with DSCG (**g**, **h**, respectively) are shown. In mice during LAR (**g**), remarkably greater amounts of mucus are observed. **d–f**, **i**, **j** Pi and mucus area (M) of the corresponding panels were marked to show how to measure these parameters.

assume that one or several factors generated from mast cells may trigger mucus hypersecretion during LAR. Alternatively, it is possible that DSCG has a direct stabilizing effect against airway epithelial cells, which are responsible for airway mucus secretion, and consequently attenuates the degranulation of these cells. Further studies are required to examine this possibility.

In conclusion, we have shown that airway mucus hypersecretion may be a component of airflow obstruction during LAR, but not during IAR, in a murine model of asthma. DSCG may be effective in reducing excessive airway mucus during asthmatic attacks in humans: further studies are required to examine this possibility.

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References

1 Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE: Allergen-induced increase in nonallergic bronchial reactivity. Clin Allergy 1977; 7:503–513.
2 Durham SR: The significance of late responses in asthma. Clin Exp Allegy 1991;21:3–7.
3 Tokuyama K, Kuo H-P, Rohde JAL, Barnes PJ, Rogers DF: Neural control of goblet cell secretion in guinea pig airways. Am J Physiol (Lung Cell Mol Physiol) 1990;259:L108–L115.
4 Shimura S, Andoh Y, Haraguchi M, Shirato K: Continuity of airway goblet cells and intraluminal mucus in the airways of patients with bronchial asthma. Eur Respir J 1996;9:1395–1401.
5 Hashimoto K, Ishii Y, Uchida Y, Kimura T, Masuyama K, Morishima Y, Hirano K, Nomura A, Sakamoto T, Takano H, Sagai M, Sekizawa K: Exposure to diesel exhaust exacerbates allergen-induced airway responses in guinea pigs. Am J Respir Crit Care Med 2001; 164:1957–1963.
6 Aikawa T, Shimura S, Sasaki H, Takishima T, Yaegashi H, Takahashi T: Morphometric analysis of intraluminal mucus in airways in chronic obstructive pulmonary disease. Am Rev Respir Dis 1989;140:477–482.
7 Cockcroft DW, Murdock KY: Comparative effects of inhaled salbutamol, sodium cromoglycate, and beclomethasone dipropionate on allergen-induced early asthmatic responses, late asthmatic responses, and increased bronchial responsiveness to histamine. J Allergy Clin Immunol 1987;79:734–740.
8 Cieslewicz G, Tomkinson A, Adler A, Dues C, Schwarze J, Takeda K, Larson KA, Lee JJ, Irvin CG, Gelfand EW: The late, but not early, asthmatic response is dependent on IL-5 and correlates with eosinophil infiltration. J Clin Invest 1999;104:301–308.
9 Dixon M, Jackson DM, Richards IM: The effect of sodium cromoglycate on lung irritant receptors and left ventricular cardiac receptors in the anesthetized dog. Br J Pharmacol 1979; 67:569–574.
10 Crossman DC, Dashwood MR, Tailor GW, Wellings R, Fuller RW: Sodium cromoglycate: evidence of tachykinin antagonist activity in the human skin. J Appl Physiol 1993;75:167–172.
11 Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, Gelfand EW: Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. Am J Crit Care Med 1997;156:766–775.
12 Ohki Y, Tokuyama K, Mayuzumi H, Sato A, Koyama H, Takizawa T, Arakawa H, Mochizuki H, Morikawa A: Characteristic features of allergic airway inflammation in a murine model of infantile asthma. Int Arch Allergy Immunol 2005;138:51–58.
13 Bates J, Irvin C, Brusasco V, Drazen J, Fredberg J, Loring S, Eidelman D, Ludwig M, Macklem P, Martin J, Milic-Emili J, Hantos Z, Hyatt R, Lai-Fook S, Leff A, Solway J, Lutchen K, Suki B, Mitzner W, Pare P, Pride N, Sly P: The use and misuse of Penh in animal models of lung disease. Am J Respir Cell Mol Biol 2004;31:373–374.
14 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 A 1999;58:129–143.
15 Hosoe H, Kaise T, Ohmori K, Isohama Y, Kai H, Takahama K, Miyata T: Mucolytic and antitussive effects of erdosteine. J Pharm Pharmacol 1999;51:959–966.
16 Ordoñez CL, Khashayar R, Wong HH, Ferrando R, Wu R, Hyde DM, Hotchkiss JA, Zyang Y, Novikov A, Dolganov G, Fahy JV: Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. Am J Respir Crit Care Med 2001;163:517–523.
17 Wills-Karp M: Immunologic basis of antigen-induced airway hyperresponsiveness. Annu Rev Immunol 1999;17:255–281.
18 Grünig G, Warnock M, Wakil AE, 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 1998; 282:2261–2263.
19 Hays SR, Woodruff PG, Khashayar R, Ferrando RE, Liu J, Fung P, Zhao CQ, Wong HH, Fahy JV: Allergen challenge causes inflammation but not goblet cell degranulation in asthmatic subjects. J Allergy Clin Immunol 2001; 108:784–790.
20 NHLBI: Global Initiative for Asthma. Global strategy for asthma management and prevention. Bethesda, National Institutes of Health, 2002, NIH Publication No. 02-3659.
21 Hutson PA, Holgate ST, Church MK: The effect of cromolyn sodium and albuterol on early and late phase bronchoconstriction and airway leukocyte infiltration after allergen challenge of nonanesthetized guinea pigs. Am Rev Respir Dis 1988;138:1157–1163.
22 Nakajima H, Iwamoto I, Tomoe S, Mastumura R, Tomioka H, Takatsu K, Yoshida S: CD4+ T-lymphocytes and interleukin-5 mediate antigen-induced eosinophil infiltration into the mouse trachea. Am Rev Respir Dis 1992;146: 374–377.
23 Cohn L, Homer RJ, MacLeod H, Mohrs M, Brombacher F, Bottomly K: Th2-induced airway mucus production is dependent on IL-4Rα, but not on eosinophils. J Immunol 1999; 162:6178–6183.

小児気管支喘息の発症パターンと原因・危険因子の多様性

Variety of onset and risk factors for childhood asthma

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Summary

小児気管支喘息は年齢によって，その原因・危険因子が異なるが，乳幼児期には，家族歴やアレルギー素因，性別などの個体側の因子の関与が大きい。その後，環境因子の関与が大きくなる。しかし，環境因子のなかでも受動喫煙や大気汚染などの因子はやはり乳幼児期の影響が強いことがわかっている。アレルギーの素因をもつ場合や男子など喘息の危険因子の強いものでは，特に環境因子に注意することが必要である。そして，小児期における危険因子は加齢とともに，徐々に成人と同様な因子の関与が強くなっていく。したがって，喘息の良好な治療やコントロールのためには小児科・内科の連携も必要である。

Key words

危険因子，小児気管支喘息，
喫煙，鼻アレルギー，環境因子

はじめに

小児気管支喘息（以下，喘息）は近年増加の傾向にあり，われわれの西日本11県の調査では，10年間隔で1.4倍に増加している。この増加の原因や危険因子は必ずしも明らかではない。また，近年報告されている疫学調査成績も今後さらに検討されねばならない点が多い。

危険因子についてGlobal Initiative for Asthma（GINA）では表1のように分類して述べられているが，ここでは，年齢別に原因・危険因子について述べてみたい。

I　喘息の危険因子

1．乳児期

1）遺伝的素因

遺伝的因子は実際の臨床上では，主に家族歴ということになる。家族歴は確かにどの年齢であっても有力な危険因子であることに間違いはない。しかし，これは妊娠後に考えても仕方のないことである。問題はこれを前提としていかにするのかである。また，現在の疫学調査では，

表1．小児気管支喘息の危険因子（GINA2002）

Ⅰ．気管支喘息の発症・増悪に関わる危険因子
1．生体因子
①アレルギーの素因と遺伝子
②気道過敏性
③性差
2．環境因子
—発症および症状増悪に関わる因子—
①アレルゲン
②ウイルスなどによる呼吸器感染
③屋外大気汚染
④室内空気汚染
⑤受動喫煙
⑥食品および食品添加物
⑦寄生虫感染
⑧運動と過換気
⑨気象
⑩薬物
⑪激しい感情表現とストレス
⑫その他
Ⅱ．出生前期・新生児期・乳児期の因子

小学校1年生の2人に1人は何らかのアレルギー疾患をもっており[1]，アレルギー素因のない人間と結婚すること自体が難しくなっているともいえよう。

乳児期の発症因子としては，まず家族歴である。喘息の両親，またはその一方から生まれた者は明らかに喘息が発症しやすい[1)-4)]。

2）男子

これも生まれてからではどうしようもないが，喘息に関しては，小児期では男子が女子の約1.7倍多いことが各報告[2]でみられている。その理由に関しては必ずしも明らかではない。

3）既往歴

患者自身が何らかのアレルギー疾患をもつものでは，ないものに比較してアレルギー疾患や喘息が発症しやすい。これは，アレルギーマーチという表現にみられるように，アトピー性皮膚炎やアレルギー性鼻炎などのアレルギー疾患をもった者に喘息が発症しやすいとして知られている。すでにアレルギー疾患がある場合には，特に環境因子に気をつけることが望ましい。

4）胎児期の状況

母親が児を妊娠中に感染やアレルギー症状があると喘息が発症しやすい。また母親が妊娠中にタバコを吸っていると臍帯血中のIgEが高く，喘息を発症しやすい。

5）出生時の状況

出生時体重が大きい者は小さい者に比べ，相対的に喘息が発症しやすいと報告されている。われわれも同様の結果を得ている[5]。

6）感染

感染によって喘息の発症はむしろ抑制されるとの考えが報告されている。しかし，全く逆の報告も多く，条件を絞った検討が必要である。また，RS（respiratory syncytial）ウイルス感染症に罹患すると喘息が発症しやすい。特にアレルギー疾患の症状がある場合には発症しやすいことがわかっている。これに関しては，喘息の家族歴のある場合に，特に発症しやすい。したがって家族歴のある場合には感染に特に注意が必要である。

7）栄養方法

母乳栄養の是非に対しても多くの報告がある。この点についても対立する結果があり，必ずしも明らかではない。

8）母親の喫煙

母親の喫煙は，喘息の発症要因として，また改善可能なはずのものとして重要である（表2）[6]。

9）その他

近年，報告されている衛生仮説からは従来の考えと異なる興味深い考えが示されている。第2子，兄弟が多い，BCG接種がない，2歳までの抗生物質の使用[7]など。しかし，日本におけるわれわれの疫学調査の結果は必ずしもこれと一致していない。今後の検討課題である。人種差や経済的因子も，全年齢で関与の可能性が推定されている[8]が，これも多くの要因を含んでいると考えられ，今後の検討課題である。

2．幼児期

3歳児を対象とした環境省のサーベイランスの結果で喘息の有症率との関係が有意なものは，表3の

表2．母親の喫煙と喘息の関係
これらの影響は男子に大きい。

1．妊娠中
①流産など異常分娩の危険（2次的に呼吸障害児の発生？）
②低出生体重児の増加（易感染→気道過敏性の獲得の可能性）
③アレルギーの易獲得性（臍帯血IgE値の上昇）
④生後早期の気道の脆弱性（易感染性，低肺機能，過敏性）

2．出産後
①気道過敏性の獲得→喘鳴，喘息の有症率の増加
1）素因者（有家族歴者，アトピー性皮膚炎児）における気道過敏性の獲得
2）非素因者における気道過敏性の獲得
3）下気道疾患易罹患性
②アレルギーの獲得，誘導
1）免疫学的に（IgE上昇など）
2）局所の障害→抗原の易侵入性
3）環境汚染→2次的にダニの増加？
③喘息患者に対する症状の誘発（母親の喫煙量に依存）
1）気道過敏性の亢進
2）換気機能の低下

3．その他
①経済的影響
②子供が将来喫煙習慣を獲得しやすい

表3．3歳児での喘息の危険因子
（ ）内の数値は平成9～14を通してのオッズ比の最小最大値

	>1.0（O.R.）	<1.0（O.R.）
6年間 （平成9～14年）	男児＞女子（1.70） 母親喫煙（1.29） 家屋構造・木造木枠（1.22） ペットあり（1.05） 昼間の保育者・保育所（1.32） 栄養法・ミルク（1.12） 栄養法・混合（1.04） 本人のアレルギー既往（2.35） 親のアレルギー既往（1.94～2.16）	SO_2（0.72） 居住歴・生まれてずっと（0.93）

（環境省環境保健部：平成14年度大気汚染に係る環境保健サーベイランス調査報告．平成16年）

ごとき項目である。

1）昼間の保育者

昼間の保育者に関しては，祖母，母，保育園の順に喘息の有症率が高い。これは感染の機会が多いためと理解されている。

2）抗原

吸入性抗原であるダニの感作は，この頃から急速に増加する。このことが気道アレルギーとしての喘息の発症と関連すると考えられる。ダニ[9]，ペット，真菌カビ，酵母なども関連するとされている[10)11)]。

3）大気汚染

大気汚染の喘息への影響は，諸々の報告がある[12)]が，全年齢で検討した場合には有意な差が認められない場合が多い。しかし，乳幼児期に限ってみれば喘息発作との関連が認められる[13)]。

4）鼻アレルギーの合併

鼻アレルギーの合併がある場合には，喘息が発症しやすいとされている。表4にその報告をまとめ

表4．鼻アレルギーと気管支喘息の関係

1．疫学的観点から

①喘息患者での鼻炎の合併

両者の合併率は一般に比べれば高いが，喘息患者の多くは鼻アレルギーを合併しているが，鼻炎が喘息を合併している率は相対的に少ない。

②上気道症状は先行して，または同時に喘息を発症する。

③鼻アレルギーのある者では，ない者に比べ喘息が発症しやすい。

④鼻アレルギーのある者では，喘息症状がなくても気道過敏性が認められる。

⑤喘息で鼻アレルギーのある者では，夜間覚醒が起きやすい。

⑥喘息で鼻炎症状のある者では抗喘息薬を多く使用する。

（特に吸入と経口のステロイド薬）

⑦鼻炎に副鼻腔炎を合併した場合には喘息を合併しやすい。

（副鼻腔炎のみでは合併は増加しないとの報告がある。）

⑧鼻炎が重症であるほど，また持続性であるほど喘息の合併率が増加する。

2．検査所見から

①鼻アレルギーで喘息の合併のない者でもメサコリン，ヒスタミンなどに対して気道過敏性を有する。

②鼻からの冷気吸入は気管支攣縮を誘発する。これは鼻粘膜の局所麻酔や抗コリン剤投与で抑制される。

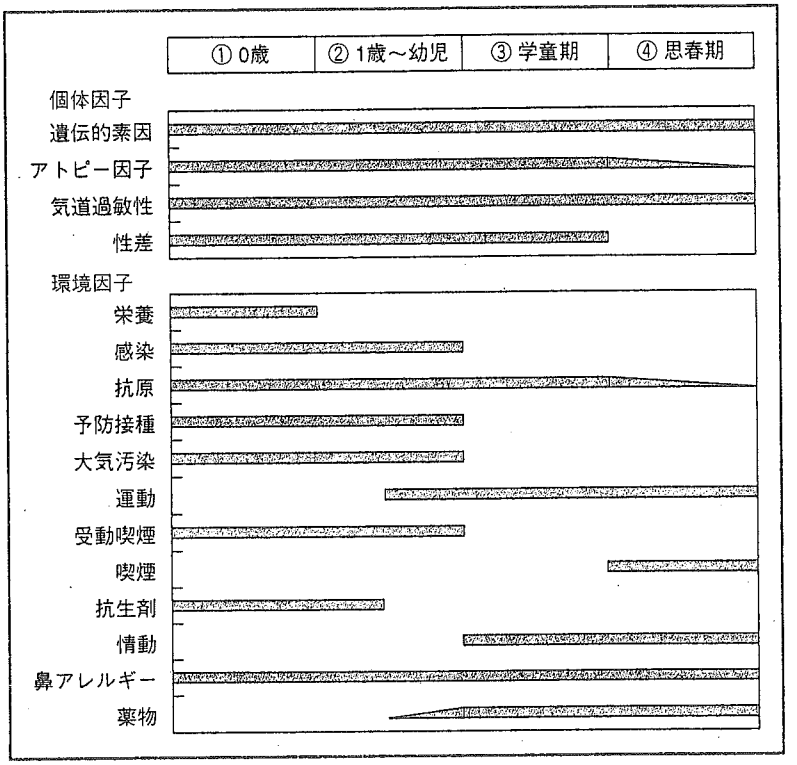

図1．年代別危険因子

た[14]。

3．学童期～思春期

この時期には，上記の2つの時期の因子に加え，以下のごとき因子が関与する。

1）第2次性徴

第2次性徴は喘息の経過を改善するとも考えられている。これはoutgrowに関しても重要である。一方で，約20%の者では喘息症状の増悪がみられる。

2）月経随伴性喘息

女子では月経と喘息の関連が認められる者もみられるようになる。

3）心因の関与

思春期を迎え，心因は喘息の原因として重要である。受験や友人関係などのストレスは喘息発作の原因となる。

4）タバコ

男子では，中学生の約10%，高校生の25%が常に喫煙しているとの報告がある。女子ではそれぞれ1%，10%である。喫煙は喘息の増悪因子であることは各種報告に示されている通りである。

5）運動誘発喘息

患児の体力に合わせた負荷量を設定して，運動負荷を実施すれば，喘息児の90%以上に運動誘発喘息を認める。これは気道の運動による冷却と脱水が主要因と考えられている。冬季のマラソンは，その典型的な誘発因子である。これは，よく観察すれば幼児でも認められる。この場合には，重症であれば運動制限をすることになるが，それでは体力がなくなり，さらに運動誘発発作が起こりやすくなる。そこで，適切な予防処置（β_2刺激薬やDSCGの直前の吸入，ロイコトリエン受容体拮抗薬の投与など）により，予防しながら運動を続けることで，起こりにくくなり，また気道の過敏性も改善する[15]。

6）薬物（アスピリン）

薬物に関しては，アナフィラキシーの形での気道狭窄は各年代に認められる。ただし，アスピリン喘息は思春期以降の女子に多い[16]。

7）食物

食物に関しては，野菜と同様に穀物やナッツ，デンプンからの蛋白，穀物と米からのカロリー摂取の低下はアレルギー疾患を増やすと考えられている[17]。

まとめ

喘息の発症には多くの因子が関与すると考えられている。各因子には，必ずしも同一の検討結果が報告されているのではなく，否定的な場合もある。ここでは，現時点での一応のまとめとして示した。また，そのおおまかなまとめを図1として示した。

文　献

1) 西日本小児アレルギー研究会・有症率調査委員会：西日本小学児童におけるアレルギー疾患有症率－1992年と2002年の比較－．日小児アレルギー会誌 17：255-268, 2003
2) 松本一郎，小田嶋博，西間三馨，他：同一地域，同一調査法による15年間のアレルギー疾患の変化．アレルギー 48：435-442, 1999
3) Sibbald B, Horn ME, Gregg I：A family study of the genetic basis of asthma and wheezy bronchitis. Arch Dis Child 55：354-357, 1980
4) Holgate ST：Genetic and environmental interaction in allergy and asthma. J Allergy Clin Immunol 104：1139-1146, 1999
5) Kawano Y, Morikawa M, Odajima H, et al：Fetel growth promotion in allergic children. Pediatr Allergy Immunol 16：354-356, 2005
6) 小田嶋博：喫煙と気管支喘息．呼吸器科 6：567-575, 2004
7) Hopkin JM：Mechanisms of enhanced prevalence of asthma and atopy in developed countries. Curr Opin Immunol 9：788, 1997
8) Litonjua AA, Carey VJ, Weiss ST, et al：Race, socioeconomic factors, and area of residence are associated with asthma prevalence. Pediatr Pulmonol 28：394-401, 1999
9) Peat JK, Tovey E, Mellis CM, et al：Importance of house dust mite and Alternaria allergens in childhood asthma；an Epidemiological study in two climatic regions of Australia. Clin Exp Allergy 23：812-820, 1993
10) O'Hollaren MT, Yunginger JW, Offord KP, et al：Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. N Engl J Med 324：359-363, 1991
11) Salvaggio J, Aukrust L：Postgraduate course

presentations. Mold-induced asthma. J Allergy Clin Immunol 68：327-346, 1981
12) Koenig JQ：Air pollution and asthma. J Allergy Clin Immunol 104：717-722, 1999
13) 小田嶋博，広瀬隆士，西間三馨：大気汚染物質（浮遊粒子状物質，二酸化窒素）と気管支喘息発作入院数との関連．アレルギー 44：160-169, 1995
14) 小田嶋博：鼻アレルギーが小児喘息に及ぼす影響．耳鼻・頭頸外科 76：125-131, 2004
15) 小田嶋博：気管支喘息と運動，学校体育について．日小児アレルギー会誌 15：263-272, 2001
16) Szczeklik A, Nizankowska E, Duplaga M：Natural history of aspirin-induced asthma. AIANE Investigators. European Network on Aspirin-Induced Asthma. Eur Repir J 16：432-436, 2000
17) Ellwood P, Asher MI, Bjorksten B, et al：Diet and asthma, allergic rhinoconjunctivitis and atopic eczema symptom prevalence；an Ecological analysis of the international study of asthma and allergies in childhood（ISAAC）data. ISAAC Phase One Study Group, Eur Respir J 17：436-443, 2001

喫煙の気管支喘息への影響

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key words：bronchial asthma，bronchial response，lung function，maternal smoking，serum IgE

はじめに

気管支喘息（以下，喘息と略す）は増加の傾向にあり，その傾向は小児において著しい．西日本での同一地域，同一対象校，同一方法での10年間隔の調査では10年間で1.4倍，20年間で2倍に増加している．増加の原因は明らかではないが，喘息の発症，発作の増悪，症状持続の危険因子の1つとして，喫煙は重要である．喫煙は喘息の環境因子の内でも最も改善が容易と考えられるものの，その実態はそう簡単ではない．

ここでは，タバコと喘息の関係についてまとめてみたい．

1．喘息の病態と喫煙

喘息の病態は気道の炎症とそれに伴う気道の過敏性と考えられている．実際の喘息における気道の病理変化としては，細胞浸潤，血管拡張，微小血管からの漏出，上皮の破壊，平滑筋の肥大，血管新生，上皮杯細胞増殖，間質コラーゲンの上皮下沈着（基底膜肥厚）などが存在する．これらの病変，病態に関する喫煙の影響は測り知れず，疫学的に関連付けられているものや基礎的研究から関連が検討されているものもあるが，未解明の部分が多い．

わが国の喫煙率は，男子が先進諸国の中では高く，60%を超えていたが急速に減少し，更に小児科医としては，高校生男子の約20%，女子の約5%が毎日喫煙しているという事実を知らなければならない．環境省による3歳児検診を利用した調査では，父親は約40%程となっている．しかし，母親は，横ばいからわずかに増加している（図1）．更に小児科医としては，高校生男子の約20%，女子の約5%が毎日喫煙しているという事実を知らなければならない．

2．喫煙の喘息への影響（表1）

1）能動喫煙の影響

(1) 症　状

喫煙は，急性の喘息発作症状を導く[2]ことが報告され，また喫煙者は非喫煙者に比べ，喘鳴を呈するものが多く，中止によって喘鳴が減少することが報告されている[3]．

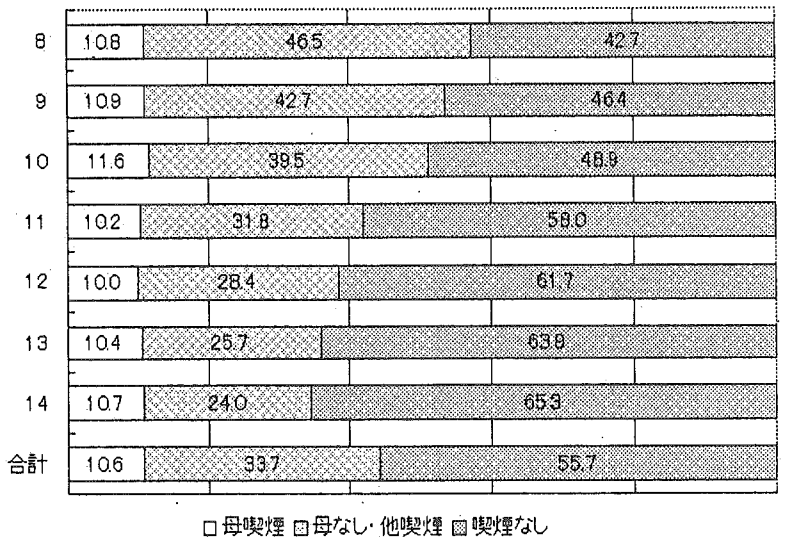

図1．平成14年度環境省調査による3歳児の家庭での喫煙率

表1. 喘息に対する喫煙の影響[1)]

1. 能動喫煙	2. 受動喫煙
1)症状	1)症状
急性発作の誘発	発作誘発
喘鳴症状を誘発*	(間接的に)下気道感染の誘発
2)肺機能	喘息有症率の上昇
肺機能を低下させる*	重症化
3)気道過敏性	2)肺機能
亢進*	低下
4)喘息発症	3)気道過敏性
家族歴がある場合は発症しやすい	亢進(乳児期>小児期)
5)予後	男女差は一定せず
・予後を悪化	4)喘息発症
・治療への反応が低下し、重症化させる	生後早期は発症因子
・肺機能の頂点の持続期間を短縮させる	5)予後
・肺の成長に悪影響	・予後を悪化
	・肺機能の不安定さ
	・長期的な肺機能の悪化

喫煙はこのほか、浮遊粒子状物質の発生源としても関与する。 *:禁煙により改善

(2) 肺機能，気道過敏性

喫煙は喘息患者の肺機能を低下させ，重症度を悪化させる[4)]，このことは，小児でも同様である[5)]．

タバコは，特に若年者の，換気機能を低下させ[6)]，また，程度はより少ないが気道の反応性に対して影響を与える．これは，喘息患者の気道の感受性が強いためであるとも推測されている．喫煙は気道の反応性を亢進するがこれは気道の炎症を起こすためとも推定されている[7)]．一方，喫煙時に見られた気道の過敏性は中止することによって減少する．また，喫煙の程度と気道過敏性の程度との間には，明らかな関連が報告されている[8)]が，高年齢になってからでも禁煙することによって肺機能の低下を低下させうることも報告されている[9,10)]．

(3) 発　症

喫煙は直接的には喘息の発症因子としては結びつかない[11)]，能動喫煙は喘息発病の危険因子であるか否かはまだ確定されていないとの報告もある[12)]．

一方で家族歴があるか，同時にまたは，親が喫煙しているかの場合には正常の乳児に比べてヒスタミンに対する反応性が強く喘息が発症しやすいと報告されている[13)]

(4) 予　後

思春期・青年期における喫煙は喘息症状の持続に関する危険因子と考えられ，小児期の喘息の25年後の予後に関する検討で喫煙は危険因子として報告[14)]されている．

予後を悪化させることは治療に対する反応を低下させることで喘息を重症化させる[15)]ことと同一と考えられる．

思春期での喫煙は，一般的に20～35歳の間にみられる$FEV_{1.0}$の頂点の持続期間を短くする[16)]．これは一般的にも喫煙の悪影響として知られているが喘息患者では更に問題となる．また，思春期からの禁煙であっても肺の成長に良好な影響を与える[9)]．

英国での調査では小児期に喘息で，その後，長期に渡り寛解を維持していた患者が33歳の時点で再発する場合は現在喫煙しているものに多い[17)]．

2) 受動喫煙

喫煙者が周囲に与えるタバコの煙（副流煙）は喫煙者本人が吸い込む煙よりも，高温で，毒性が強く気道粘膜への刺激性も強い．含まれる刺激物質の濃度は数倍～数十倍に及ぶ．受動喫煙と小児の喘息に関しては多くの報告がある．すでに1950年にタバコの除去によってやっと管理が可能になった小児例が報告されて