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Original Article

Influence of local antigen exposure dose in the upper respiratory tract on sensitization with cedar pollen

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Abstract

Background: An increase in Japanese cedar pollen counts is the probable reason for the increase in the number of Japanese cedar hay fever patients. To determine whether local antigen exposure dose affects sensitization with cedar pollen, we compared serum levels of specific IgE antibody in rats exposed to higher and lower doses of cedar pollen antigen through the nose.

Methods: Serum levels of cedar pollen-specific IgE antibody was examined in Brown Norway rats exposed to a higher dose of (20 μg) Cry j I, a lower dose (2 μg) of Cry j I or no dose for 6 months. Serum levels of cedar pollen-specific IgE antibody were measured by reverse IgE-capture ELISA. The extent of local eosinophilia in the nasal, laryngeal and tracheal mucosa of rats exposed higher and lower doses of cedar pollen antigen and controls were observed microscopically.

Results: The mean serum levels of specific IgE antibody in rats exposed to the higher dose were significantly higher than those in rats exposed to the lower dose, and the mean levels in rats in the lower-dose group were significantly higher than in controls. The extent of eosinophilia in the nasal mucosa in the higher-dose group was significantly greater than in controls, but no significant differences between the lower-dose group and controls were found. The extent of eosinophilia in the laryngeal mucosa in the higher-dose group was significantly greater than that in the lower-dose group and in controls. Only a small degree of eosinophilia was observed in the trachea of all three groups.

Conclusions: Local exposure dose of the upper airway to cedar pollen may affect sensitization.

Key words: cedar pollen, IgE, laryngeal allergy, pollinosis.

Introduction

An increase in ambient antigen is one of the most probable reasons for the increase in allergic disorders, such as allergic rhinitis, bronchial asthma and atopic dermatitis. In Japan, in recent decades the number of Japanese cedar (*Cryptomeria japonica*) hay fever patients has increased[1] and an increase in Japanese cedar pollen counts is a probable principal cause.[2] However, environmental pollution[3–6] and a westernized lifestyle, including food, house structure and construction materials, provide other possibilities for the increase in hay fever patients. There have been no conclusive reports that determine whether local antigen exposure dosage affects human sensitization with cedar pollen. Therefore, in the present study, we have examined serum levels of cedar pollen-specific IgE antibody in rats exposed to higher and lower doses of cedar pollen antigen pernasally. Furthermore, to determine the existence of laryngeal allergy to cedar pollen,[5,7] the grade of local eosinophilia in the nasal, laryngeal and tracheal mucosa of rats exposed to higher

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and lower doses of cedar pollen antigen was observed microscopically.

METHODS

Eight Brown Norway (BN) rats were exposed to 20 μg Cry j I in solution by topical application to the nasal mucosa; Cry j I is one of the major allergens of Japanese cedar pollinosis.[8] Eight BN rats were exposed in a similar manner to 2 μg Cry j I. Exposures were performed on all 16 rats 5 days/week for 6 months. Rats were then immunized twice on day 0 and day 13 with intraperitoneal injections of 10 μg Cry j I with 4.5 mg aluminum hydroxide gel as boosters in confirmed sensitization. Seven days after the last immunization, serum levels of anti-Cry j I IgE antibody were determined by reverse IgE-capture ELISA.[9] After determining the serum levels of IgE, hematoxylin and eosin-stained nasal, laryngeal and tracheal mucosa of these rats were examined microscopically. Each specimen was obtained 6 h after pernasal exposure to 10 μg Cry j I and eosinophilia in the mucosa of the whole specimen was assessed as an indicator of local allergic reaction, according to the three ranks of infiltration, which were nil (–), slight (+) and marked (++). Five non-exposed and non-sensitized rats were used as controls.

The unpaired *t*-test and χ^2-test were used to determine statistical significance and $P < 0.05$ was considered to be statistically significant.

RESULTS

Serum levels of specific IgE antibody to Cry j I immediately before immunization with intraperitoneal injection were not detected in the either higher- or lower-dose antigen-exposed rats, although these rats were exposed nasally to several doses of Cry j I for 6 months. Mean serum levels of specific IgE antibody after immunization with intraperitoneal injection were 307.1 ± 185.3 free units (FU)/mL in the higher-dose rats, 87.8 ± 169.5 FU/mL in the lower-dose group and 0.0 ± 0.0 FU/mL in the controls. There were significant differences between the higher- and lower-dose groups and the lower-dose group and the controls (unpaired *t*-test), as shown in Fig. 1.

The extent of eosinophilia of the nasal mucosa in the higher-dose group was significantly greater than in controls, but no significant differences were found between the higher- and lower-dose groups or between the lower-dose group and controls (χ^2-test), as shown in Table 1.

The extent of eosinophilia of the laryngeal mucosa in the higher-dose group was significantly greater than that in the lower-dose group and in the controls, but there were no significant differences between the lower-dose group and the controls (χ^2-test), as shown in Table 2. Only small accumulations of eosinophils were observed in the tracheal mucosa of the three groups (χ^2-test) and no significant differences were found between the three groups, as shown in Table 3.

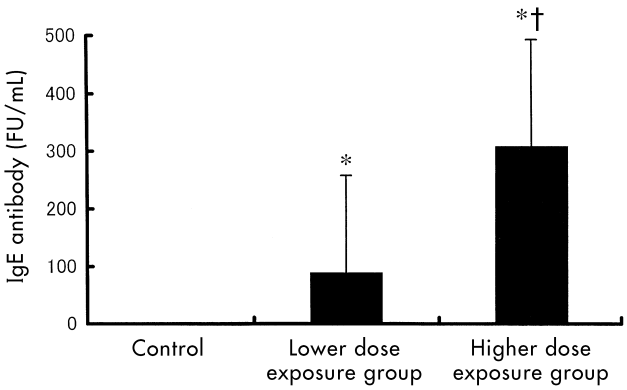

Fig. 1 Serum levels of specific IgE antibody to Cry j I in sensitized rats. *$P < 0.01$ compared with controls; †$P < 0.01$ compared with the lower-dose exposure group.

Table 1 Tissue eosinophilia in the nasal mucosa of rats sensitized with Cry j I and controls

	Grade of eosinophilia		
	–	+	++
Control ($n = 5$)	4	1	0
Lower-dose exposure group ($n = 8$)	5	2	1
Higher-dose exposure group ($n = 8$)	1	2	5

There was a significant difference ($P < 0.05$) between the higher-dose exposure group and control, but not between the lower-dose exposure group and control or between the lower- and higher-dose exposure groups.

Table 2 Tissue eosinophilia in the laryngeal mucosa of rats sensitized with Cry j I and controls

	Grade of eosinophilia		
	–	+	++
Control ($n = 5$)	4	1	0
Lower-dose exposure group ($n = 8$)	5	1	2
Higher-dose exposure group ($n = 8$)	0	5	3

There was a significant difference ($P < 0.05$) between the higher-dose exposure group and the lower-dose exposure group and controls, but not between the lower-dose exposure group and control.

Table 3 Tissue eosinophilia in the tracheal mucosa of rats sensitized with Cry j I and controls

	Grade of eosinophilia		
	–	+	++
Control ($n = 5$)	5	0	0
Lower-dose exposure group ($n = 8$)	7	1	0
Higher-dose exposure group ($n = 8$)	8	0	0

There were no significant differences between any of the three groups.

No accurate sneezing, rhinorrhea, wheezing or dyspnea in rats was observed during the period of pernasal Cry j I solution exposure.

Discussion

In Japan, Japanese cedar and cypress (*Chamaecyparis obtusa*) trees have been planted for industrial use under the direction of the Japanese government since the end of the Second World War. As a result, cedar and cypress comprise more than 46% of forest in Japan, which probably accounts for the increasing airborne pollen counts of Japanese cedar.[2] Imaoka *et al*.[10] reported that higher cedar pollen counts were associated with an increase in the number of cedar hay fever patients, but no proposed studies concerned with relationships between local antigen exposure dose in the upper respiratory tract and the extent of sensitization with cedar pollen have been reported. In the present study, it was demonstrated that higher local exposure of rats to Cry j I increased sensitization to cedar pollen. We used Cry j I as the cedar pollen antigen in the present study because we were able to obtain only Cry j I protein from natural Japanese cedar pollen. The extent of eosinophilia of the nasal mucosa in the higher-antigen dose group was significantly greater than in controls, but no significant differences in eosinophilia in the nasal mucosa between the lower-dose group and controls were found. We proved the thesis experimentally, but before we draw any conclusions, we must discuss whether local exposure of rats to doses of 2 and 20 μg/day Cry j I are high or not. However, we tried to set up the Cry j I doses in the present study according to our previous investigation.[7,11]

In recent years, laryngeal allergy to cedar pollen has been observed[5,7,11] and seasonal alterations in airborne cedar pollen counts are related with the severity of laryngeal symptoms.[5] To determine whether local antigen doses also affect allergic reactions of the laryngeal mucosa of rats, we observed local eosiniphilia of the larynx in the present study. Local exposure to antigen influenced the extent of laryngeal eosinophilia, whereas, in the trachea, only a small degree of eosinophilia was observed in any of the three groups. The differences in these results between these organs may be caused by the spread of Cry j I solution from the nose. Another reason for the differences may result from specificity of allergic reactions among the three organs. This study has suspected that the local exposure dose affects sensitization with cedar pollen and results in allergic reactions in the nasal and laryngeal mucosa, but not the trachea.

Intraperitoneal injection with Cry j I was used as the method of sensitization with cedar pollen after local exposure because in past animal studies only local antigen exposure failed to sensitize animals.[12] When rats are immunized with peritoneal injections before local antigen exposure, serum levels of specific IgE will be elevated to such an extent that we cannot assess the influences of local antigen exposure dose. Therefore, we used local antigen exposure before intraperitoneal immunization. The same dose of intraperitoneal boosters was used in both the higher- and lower-dose groups of rats. Consequently, the difference in specific IgE levels between these two groups seemed to be due to local exposure doses of Cry j I.

Allergic diseases may also be concerned with hereditary factors. Matsushita *et al*.[13] considered the human leukocyte antigen system (HLA)-DQW3 as an important hereditary indicator of Japanese cedar pollinosis. Furthermore, environmental air pollution[3–6] and a westernized lifestyle have been suggested as possibilities for the increase of allergic respiratory tract disorders in the population. It appears that allergic disorders are exacerbated with higher dose antigen exposure, but hereditary factors and other circumstantial conditions may also be involved.

Our animal studies support the view that an increase in the Japanese cedar pollen count is one of primary reasons for the increase in the number of cedar hay fever patients. However, it is difficult to come to a definite conclusion about the human phenomenon on the basis of animal-sensitizing experiments. Further investigations must be undertaken to prove this link in humans.

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References

1 Ukai K, Hirata S, Kimura T, Sakakura Y. Epidemiological study of allergic rhinitis in different districts. *Jpn. J. Allergol.* 1998; **47**: 420–5 (in Japanese).

2 Saito Y. Airborne pollen survey of *Cryptomeria japonica* and cupressaceae in Yushima, Bunkyo-ku, Tokyo in 1996 and past 20 years (1977–96). *Jpn. J. Palynol.* 1996; **42**: 149–53 (in Japanese).

3 Saxson A, Zhang K, Takenaka H, Casillas A, Dias-Sanches D. The role of diesel exhaust in the allergic antibody response of the human airway. *J. Jpn. Immunol. Allergol. Otolaryngol.* 1996; **12**: 9–11.

4 Naito K, Ishii G, Ogawa T, Yokoyama N, Iwata S. Specific IgE and IgG4 antibodies to Japanese cedar pollen and total antibody in lumbermen. *Aerobiologia* 1998; **14**: 321–4.

5 Naito K, Iwata S, Yokoyama N. Laryngeal symptoms in patients exposed to Japanese cedar pollen: Allergic reactions and environmental pollution. *Eur. Arch. Otorhinolaryngol.* 1999; **256**: 209–11.

6 Matsumura T. The effect of ozone, nitrogen dioxide on the experimentally induced allergic respiratory disorders in guinea pigs. I. The effects on sensitization with albumin through the airway. *Am. Rev. Respir. Dis.* 1970; **102**: 430–7.

7 Ishii J, Ogawa T, Naito K *et al*. Local eosinophilia of the nose, the larynx and the trachea in rats sensitized with Japanese cedar pollen. *Jpn. J. Allergol.* 1997; **46**: 1251–7 (in Japanese).

8 Yasueda H, Yui Y, Shimizu T, Shida T. Isolation and partial characterization of the major allergen from Japanese cedar (*Cryptomeria japonica*) pollen. *J. Allergy Clin. Immunol.* 1983; **71**: 77–86.

9 Sakaguchi M, Inoue S, Taniai M, Ando S, Matuhasi T. Identification of the second major allergen of Japanese cedar pollen. *Jpn. J. Allergol.* 1990; **45**: 309–12.

10 Imaoka K, Miyazawa H, Nishihara S, Sakaguchi M, Inouye S. Effect of pollen exposure on serum IgE and IgG antibody responses in Japanese cedar pollinosis patients. *Allergol. Int.* 1996; **45**: 159–62.

11 Naito K, Baba R, Ishii G, Yokoyama N, Ibata K. Laryngeal allergy: A commentary. *Eur. Arch. Otorhinolaryngol.* 1999; **256**: 455–7.

12 Muranaka M, Suzuki S, Koizuki K *et al*. Adjuvant activity of deisel-exhaust particulates for the production of IgE antibody in mice. *J. Allergy Clin. Immunol.* 1986; **77**: 616–23.

13 Matsushita S, Muto M, Suemura M, Saito Y, Sasazuki T. HLA-linked non-responsiveness to *Cryptomeria japonica* pollen antigen. Non-responsiveness is mediated by antigen-specific suppressor T cell. *J. Immunol.* 1987; **138**: 109–15.