ORIGINAL ARTICLE

Serum Interleukins 12 and 18 and Immunoglobulin E Concentrations and Allergic Symptoms in Japanese Schoolchildren

M Ando,[1] M Shima[2]

[1] Department of Respirology, Graduate School of Medicine, Chiba University, Chiba, Japan
[2] Department of Public Health, Hyogo College of Medicine, Nishinomiya, Japan

Abstract

Background: There is considerable concern about the rising trend in the prevalence of asthma and allergic diseases. The ability to monitor this trend would be enhanced by the use of a biological marker for these diseases.

Objective: This study investigated whether serum interleukin (IL) 12 and IL-18 levels were associated with allergic symptoms such as those of asthma, allergic rhinitis, and atopic eczema in Japanese schoolchildren.

Methods: Allergic symptoms and serum IL-12, IL-18, and immunoglobulin (Ig) E levels were examined in 370 schoolchildren aged 9-10 years living in urban Japanese areas. Allergic symptoms were assessed with a questionnaire designed in accordance with the protocol of the International Study of Asthma and Allergies in Childhood (ISAAC).

Results: Serum IL-12 levels in children were not associated with any allergic symptoms. However, serum IL-18 levels were significantly higher in children who had asthma, allergic rhinitis, or atopic eczema than in those who did not have such symptoms. Serum IL-18 levels were also significantly higher in children with IgE levels of 250 IU/mL or above than in those with levels below 250 IU/mL. Gender-adjusted serum IL-18 levels were still significantly higher in children with allergic rhinitis, atopic eczema, or at least one allergic symptom than in those without symptoms.

Conclusion: These results suggest that serum IL-18 levels are associated with allergic symptoms in children, independent of serum IgE levels. Thus, serum IL-18 may be a useful biological marker of these diseases.

Key Words: Interleukin 12, IL-12. Interleukin 18, IL-18. Immunoglobulin E, IgE. Asthma. Allergic rhinitis. Atopic eczema. Allergy. Children.

Resumen

Antecedentes: Existe una considerable preocupación por la tendencia creciente en la prevalencia del asma y enfermedades alérgicas. La capacidad de vigilar esta tendencia mejoraría con el uso de un marcador biológico para estas enfermedades.

Objetivo: En este estudio se investigó si los niveles séricos de interleucina (IL) 12 e IL-18 tenían una relación con los síntomas alérgicos como los del asma, la rinitis alérgica y el eccema atópico entre los escolares japoneses.

Métodos: Se examinaron los síntomas alérgicos y los niveles séricos de inmunoglobulina (Ig) E, IL-12 e IL-18 en 370 escolares de 9 a 10 años de edad de zonas urbanas japonesas. Se valoraron los síntomas alérgicos con un cuestionario diseñado según el protocolo del Estudio Internacional del Asma y Alergias en la Infancia (ISAAC).

Resultados: Los niveles séricos de IL-12 en niños no se asociaron con ningún síntoma alérgico. Sin embargo, los niveles séricos de IL-18 se encontraron significativamente más elevados en niños con asma, rinitis alérgica o eccema atópico que en los niños que no presentaban estos síntomas. Los niveles de IL-18 también se encontraron significativamente más elevados en niños con niveles de IgE de 250 UI/mL o superiores que en aquellos con niveles por debajo de las 250 UI/mL. Los niveles séricos de IL-18 distribuidos por sexo fueron significativamente superiores entre los niños con rinitis alérgica, eccema atópico o con un síntoma alérgico, como mínimo, comparados con los de los niños asintomáticos.

Conclusión: Estos resultados permiten suponer que los niveles séricos de IL-18 en niños están relacionados con los síntomas alérgicos, independientemente de los niveles séricos de IgE. Por consiguiente, la IL-18 sérica puede ser un marcador biológico útil de estas enfermedades.

Palabras clave: Interleucina 12, IL-12. Interleucina 18, IL-18. Inmunoglobulina E, IgE. Asma. Rinitis alérgica. Eccema atópico. Alergia. Niños.

Introduction

Studies have shown that the prevalence of asthma and allergic diseases is increasing in Western and developing countries [1-3]. The International Study of Asthma and Allergies in Childhood (ISAAC), designed to facilitate comparisons among populations in different countries, reported that there are significant worldwide variations in the prevalence of allergic diseases [2,3].

Elevated levels of immunoglobulin (Ig) E have been reported to be associated with atopy and bronchial hyperresponsiveness [4]. However, Burney et al [5] showed that there were substantial variations in the prevalence of atopy and in serum IgE concentrations. These findings point to the need for a more specific biological marker with which to evaluate allergic disease. Subsequently, we found that serum levels of soluble intercellular adhesion molecule-1 were significantly higher in asthmatic children [6].

Recently, great progress has been made in elucidating the role of helper T (T_H) cells in allergic diseases. Based on their cytokine profiles, two forms of T_H cells have been identified in humans and mice [7]. Type 1 (T_H1) cells synthesize interferon (IFN) γ, inhibit IgE responses, and block the development of type 2 (T_H2) cells. T_H2 cells produce interleukin (IL)-4 and IL-13, which stimulate the production of IgE [8]. IL-18 has been identified as a proinflammatory cytokine [9] that is expressed in T_H1-mediated chronic inflammatory diseases. IL-18, along with IL-12, is known to be a potent inducer of IFN-γ production, and their coadministration has been shown to inhibit IgE production [10]. IL-12 is characterized as a heterodimeric cytokine that induces cell-mediated immune functions, upregulates T_H1 cytokines, and inhibits or downregulates T_H2 cytokines [11]. Together, IL-18 and IL-12 are known to be potent inducers of T_H1 cell-mediated responses, although IL-18 can also induce T_H2 cytokines and IgE production, and crossregulates T_H1 and T_H2 responses [12].

This study investigated whether serum IL-12 and IL-18 levels in Japanese schoolchildren were associated with symptoms of asthma, allergic rhinitis, and atopic eczema.

Materials and Methods

Subjects and Data Collection

We studied 609 children in the fourth grade (age bracket, 9-10 years) who attended 6 elementary schools located in urban areas of Chiba prefecture, Japan, in 1998 and 1999.

In September 1998 and 1999, a questionnaire designed in accordance with the ISAAC protocol [13,14] was distributed to all subjects by their schools. The questionnaire, consisting of questions concerning symptoms of asthma, allergic rhinitis, and atopic eczema, was completed by either the subject's parents or guardians; improperly completed questionnaires, accompanied by a request for completion, were returned to the subjects in order to obtain complete information. Children who reported wheezing or whistling in the chest in the 12 months prior to the study were defined as having asthma. Children who, in the 12 months prior to the study, had had sneezing or a runny or blocked nose when they did not have a cold or the flu were considered to have allergic rhinitis. Children were considered to have atopic eczema if they answered affirmatively to all of the following questions: Have you ever had an itchy rash which came and went for at least 6 months? If so: Have you had this itchy rash at any time in the last 12 months? If so: Has this itchy rash at any time affected any of the following spaces: the folds of the elbows, behind the knees, the front of the ankles, the folds under the buttocks, or the skin around the neck, ears, or eyes? Children who had at least 1 allergic symptom related to asthma, allergic rhinitis, or atopic eczema were considered to have an allergy.

From October 1998 to February 1999, and from October 1999 to January 2000, after written consent was obtained from their respective parents or guardians, venous blood samples were collected from the subjects for whom a completed questionnaire was available. Blood samples were centrifuged on the day they were collected and assayed for IgE levels; the remaining sera were stored at –80°C until IL-12 and IL-18 assays were done. Serum IL-12 and IL-18 levels were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits for human IL-12 (Endogen Inc, Woburn, Massachusetts, USA) and human IL-18 (Medical & Biological Laboratories Co Ltd, Nagoya, Japan). The minimum detection levels for IL-12 and IL-18 were 25.6 pg/mL and 12.5 pg/mL, respectively. Serum IgE levels were measured using a Behring Nephelometer Analyzer (Dade Behring, Marburg, Germany).

The study protocol was approved by the ethics committee of the Graduate School of Medicine, Chiba University.

Data Analysis

Serum IL-12, IL-18, and IgE levels had an approximately log-normal distribution. Therefore, values were converted to logarithms, and the results were expressed as geometric means with 95% confidence intervals and analyzed by gender, asthma, allergic rhinitis, atopic eczema, and at least 1 allergic symptom.

Based on the criterion accepted for Japanese children [15], we classified a serum IgE level of 250 IU/mL or more as high, and considered such an individual to have an atopic predisposition. In this study, serum IL-12 and IL-18 levels were compared between children with serum IgE levels of 250 IU/mL or more and those with serum IgE levels less than 250 IU/mL. The geometric means of the IL-12 and IL-18 levels were adjusted for gender using the general linear model and compared relative to allergic symptoms. For 2 subjects for whom the serum IL-12 concentrations were undetectable, the minimum detection limit of the assay was used in the statistical analysis. Serum IL-18 levels were detected in all cases.

Statistical analyses were performed using SYSTAT (SYSTAT Software, Richmond, California, USA).

Results

Blood samples were collected from 167 children in 1998 and from 206 more children in 1999, for a total of 373

Table 1. Serum IL-12, IL-18, and IgE levels in children by gender and allergic symptoms*

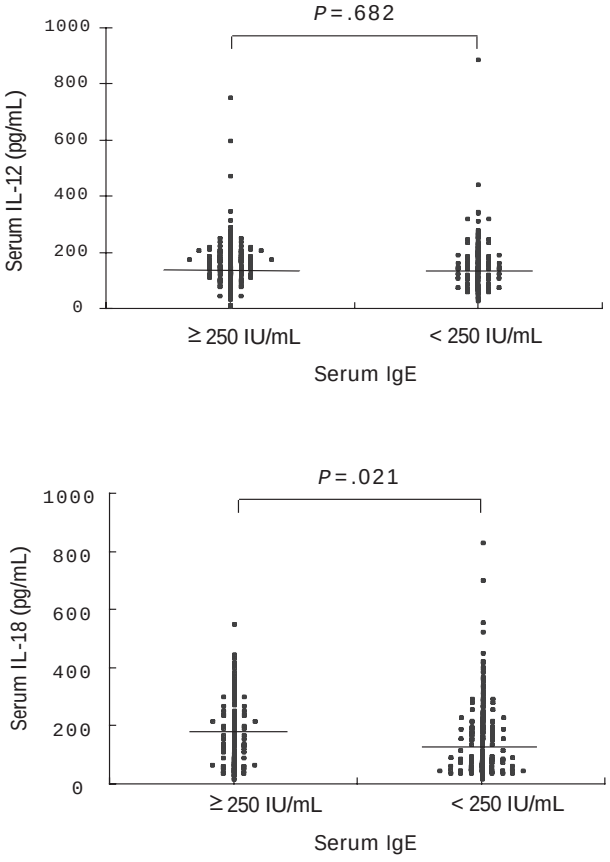
	No.	IL-12 (pg/mL) GM (95% CI)	IL-18 (pg/mL) GM (95% CI)	IgE (IU/mL) GM (95% CI)
Gender				
Male	224	146.9 (136.6–158.1)	145.5 (131.9–160.5)	148.4 (115.0–191.5)
Female	146	141.2 (129.0–154.5)	119.1 (105.3–134.7)	90.0 (65.6–123.5)
P value		.597	.015	.018
Asthma				
Yes	84	149.9 (133.0–169.0)	154.5 (131.5–181.5)	492.7(335.0–724.9)
No	286	142.6 (133.6–152.2)	129.0 (53.5–140.9)	80.6 (65.4–99.5)
P value		.536	.048	<.001
Allergic rhinitis				
Yes	98	144.0 (129.0–160.8)	152.9 (131.8–177.5)	230.4 (157.6–337.0)
No	272	145.5 (136.0–155.6)	127.7 (116.7–139.8)	96.5 (76.9–121.1)
P value		.863	.040	<.001
Atopic eczema				
Yes	52	135.6 (116.6–157.7)	164.0 (133.5–201.5)	376.2 (225.9–626.4)
No	318	145.5 (116.6–157.7)	129.0 (118.9–140.1)	101.5 (82.3–125.2)
P value		.378	.035	<.001
At least 1 allergic symptom				
Yes	173	145.5 (134.0–157.9)	156.0 (139.5–174.5)	304.9 (234.4–396.6)
No	197	144.0 (133.2–155.7)	116.7 (105.2–129.5)	54.6 (42.7–69.8)
P value		.880	<.001	<.001

*All data are geometric means (GM) followed by the 95% confidence interval (CI). IL indicates interleukin; Ig, immunoglobulin.

Table 2. Gender-adjusted serum IL-12 and IL-18 levels (pg/mL) in children, by allergic symptoms*

	No.	IL-12 (pg/mL) GM (95% CI)	IL-18 (pg/mL) GM (95% CI)
Asthma			
Yes	84	148.6 (131.5–167.9)	148.9 (126.3–175.5)
No	286	142.9 (133.9–152.6)	126.7 (116.0–138.4)
P value		.577	.087
Allergic rhinitis			
Yes	98	142.9 (127.9–159.8)	150.3 (129.4–174.5)
No	272	144.6 (135.1–154.7)	124.9 (114.1–136.8)
P value		.865	.037
Atopic eczema			
Yes	52	135.4 (116.3–157.7)	162.1 (132.1–198.8)
No	318	145.6 (136.8–155.1)	126.7 (116.4–137.8)
P value		.385	.029
At least 1 allergic symptom			
Yes	173	144.7 (133.0–157.5)	152.5 (136.3–170.6)
No	197	143.6 (132.8–155.4)	115.2 (103.8–127.9)
P value		.900	<.001

*All data are geometric means (GM) followed by the 95% confidence interval (CI) calculated by general linear models and adjusted for gender. IL indicates interleukin; Ig, immunoglobulin.



Serum interleukin (IL) 12 and IL-18 levels in children, by serum immunoglobulin (Ig) E concentrations. Horizontal bars represent the geometric mean adjusted for gender, calculated by the general linear model.

subjects (61.2% of the original 604 subjects). Questionnaires were collected from all of these children. Three children were excluded because they had symptoms, such as cold or fever, on the day of sampling or the day prior to sampling. Thus, the data from 370 children (60.8%) were analyzed.

Serum IL-12, IL-18, and IgE levels are shown in Table 1 by gender and the presence of asthma, allergic rhinitis, atopic eczema, and at least one allergic symptom. IL-18 and IgE levels were significantly higher in boys than in girls. IL-18 and IgE levels were significantly higher in subjects who had asthma, allergic rhinitis, atopic eczema, or at least 1 allergic symptom than in those without these symptoms. There was no relationship between serum IL-12 levels and allergic symptoms.

Serum IL-18 levels were significantly higher in children with high IgE levels than in children with low IgE levels. However, no significant difference was found in the serum IL-12 levels in function of IgE level (figure).

Table 2 shows the gender-adjusted geometric mean serum IL-12 and IL-18 levels by the presence of allergic symptoms. Serum IL-18 levels continued to be significantly higher in children with allergic rhinitis, atopic eczema, or at least 1 allergic symptom than in those without such symptoms. In children who had asthma, serum IL-18 levels were also higher than in those without asthma, but the differences were not significant. There were no differences in serum IL-12 levels in relation to presence of allergic symptoms.

Discussion

In this study, we examined the relationship between allergic symptoms and IL-12, IL-18, and IgE levels in schoolchildren living in urban Japanese areas.

IL-12 induces cell-mediated immunity by upregulating T_H1 cytokines, especially IFN-γ, which inhibit both IgE production and the eosinophil recruitment associated with allergic diseases [16]. IL-18, identified as an IFN-γ–inducing factor, is a proinflammatory cytokine that plays an important role in T_H1 cell activation [9]. Both of these cytokines have also been shown to affect T_H2 function. IL-12 has been shown to directly inhibit T_H2 cytokine function by an IFN-γ–independent mechanism. Gavett et al [16] demonstrated in a mouse model that IL-12 effectively suppressed inflammatory airway hyperresponsiveness that was induced by repeated antigen challenge.

Recent studies have shown that IL-18 may have the potential to act as a T_H2 cell promoting factor in atopy. Elevated serum concentrations of IL-18 have been reported in patients with asthma, allergic rhinitis, and atopic dermatitis [17-19]. Kumano et al [20] reported that IL-18 enhanced antigen-induced eosinophil recruitment into the airways of ovalbumin-sensitized mice. Wild et al [21] showed that the administration of IL-18, in conjunction with allergic sensitization and allergen challenge, increased serum IgE and IgG1 levels and increased IL-4 and IL-5 production by mouse splenocytes cultured with ragweed. Finally, Yoshimoto et al [22] reported that the injection of both IL-18 and IL-12 increased IgE levels in helminth-infected, IFN-γ–deficient mice and upregulated IL-4 and IL-13 production by basophils. Furthermore, the injection of IL-18 alone enhanced basophil IL-4 and histamine production in both wild-type and IFN-γ–deficient mice [22]. Given these results, it was deduced that IL-18 may act as a coinducer of both T_H1 and T_H2 cytokines.

Bronchial asthma is a T_H2 cytokine-dominant disease, in which the production of T_H1 cytokines, such as IL-12, is suppressed [23]. However, the role of cytokines in asthma is not conclusive [24]. Tanaka et al [17] reported finding significantly elevated serum IL-18 levels in acute asthmatic patients, and these levels correlated with serum soluble IL-2 receptor levels but not with IFN-γ levels.

In this study, serum IL-18 levels in children with asthma, allergic rhinitis, atopic eczema, or at least 1 allergic symptom were higher than in children without these symptoms, while serum IL-12 levels were comparable in allergic and non-allergic children. These data suggest that IL-18 may be related to the pathophysiological mechanisms of allergic disease. Furthermore, we found that children with high IgE levels had significantly higher serum IL-18 concentrations, suggesting that IL-18 may upregulate IgE levels, perhaps through an IFN-γ–independent pathway. Given that this was a cross-sectional study, longitudinal studies should now be done to clarify the role of IL-18 in the development of allergic diseases.

In children, an elevated serum IgE level reflects an atopic predisposition [4]. Nonetheless, many children who have low IgE levels are symptomatic [25]. In this study, the geometric means of IL-18 levels, adjusted for gender, were still significantly higher in children with allergic symptoms than in those without symptoms. This finding lends further support to the hypothesis that IL-18 has a role in allergic disease that is independent of serum IgE levels. Recent studies have suggested that genetic polymorphisms of IL-18 were also associated with atopy and asthma [26,27]. Thus, IL-18 appears to play a crucial role in the pathogenesis of allergic diseases.

The major differences in the prevalence of allergic diseases that were found among populations in ISAAC are probably due to environmental factors. A number of epidemiological studies have reported associations between urbanization and a high prevalence of allergy [31-33]. Air pollution caused by high traffic flow has been shown to either exacerbate allergic symptoms or to reduce the dose of allergen required to evoke an allergic response in persons with already existing allergic disease [28]. Sichletidis et al [29] observed that particulate pollution was associated with a rising prevalence of allergic rhinitis and low nasal flow. Wyler et al [30] showed that living on busy roads was associated with a higher risk for sensitization to pollen, a finding which suggests that a sensitizing interaction between pollen and air pollutants may occur. The use of biochemical markers may assist in the evaluation of the role of air pollutants in allergy [6,15]. While no differences in serum IgE levels were found in relation to the air pollution level in Japanese schoolchildren [15], it remains to be seen whether, in light of our results, there is an association with IL-18 levels. Further studies are needed to evaluate whether serum IL-18 is useful as a new biological marker for allergic diseases.

In conclusion, our study showed that, in children living in urban Japanese areas, serum IL-18 levels were associated with allergic symptoms, independently of serum IgE levels. Serum IL-18 concentration might be a candidate biological marker for the evaluation of environmental factors such as air pollution affect allergic diseases. The association between environmental factors and IL-18 needs further investigation in broader populations, including those living in rural areas.

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References

1. Von Mutius E. The rising trends in asthma and allergic disease. Clin Exp Allergy. 1998;28(Suppl 5):45-9.
2. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). Eur Respir J. 1998;12:315-35.
3. Asher MI, Weiland SK. The International study of asthma and allergies in childhood (ISAAC). Clin Exp Allergy. 1998;28(Suppl.5):52-66.
4. Marsh DG. Genetic and molecular analysis of human immune responsiveness to allergens. In: Marsh DG, Lockhart A, Holgate ST, editors. The genetics of asthma. Oxford: Blackwell Scientific Publications; 1993. p. 201-13.
5. Burney P, Malmberg E, Chinn S, Jarvis D, Luczynska C, Lai E. The distribution of total and specific serum IgE in the European Community Respiratory Health Survey. J Allergy Clin Immunol. 1997;99:314-22.
6. Ando M, Shima M, Adachi M, Tsunetoshi Y. The role of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and regulated on activation, normal T-cell expressed and secreted (RANTES) in the relationship between air pollution and asthma among children. Arch Environ Health. 2001;56:227-33.
7. Street NE, Mosmann TR. Functional diversity of T lymphocytes due to secretion of different cytokine patterns. FASEB J. 1991;5:171-7.
8. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR, Kay AB. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med. 1992;326:298-304.
9. Okamura H, Tsutsui H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K, Akita K, Namba M, Tanabe F, Konishi K, Fukuda S, Kurimoto M. Cloning of a new cytokine that induced IFN-γ production by T cells. Nature. 1995;378:88-91.
10. Yoshimoto T, Okamura H, Tagawa Y, Iwakura Y, Nakanishi K. Interleukin 18 together with interleukin 12 inhibits IgE production by induction of interferon−γ production from activated B cells. Proc Natl Acad Sci USA. 1997;94:3948-53.
11. Trinchieri G. Interleukin-12: A Proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Ann Rev Immunol. 1995;13:251-76.
12. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. Annu Rev Immunol. 2001;19:423-74.
13. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Miotchell EA, Pearce N, Sibbald B, Stewart AW, Strachan D, Weiland SK, Williams HC. International study of asthma and allergies in childhood (ISAAC): rationale and methods. Eur Respir J. 1995;8:483-91.
14. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis and atopic eczema: ISAAC. Lancet. 1998;351:1225-32.
15. Shima M, Adachi M. Serum immunogloblin E and hyaluronate levels in children living along major roads. Arch Environ Health. 1996;51:425-30.
16. Gavett SH, O'Hearn D, Li X, Huang SK, Finkelman FD, Wills-Karp M. Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. J Exp Med. 1995;182:527-36.
17. Tanaka H, Miyazaki N, Oashi K, Teramoto S, Shiratori M, Hashimoto M, Ohmichi M, Abe S. IL-18 might reflect disease activity in mild and moderate asthma exacerbation. J Allergy Clin Immunol. 2001;107:331-6.
18. Verhaeghe B, Gevaert P, Holtappels G, Lukat KF, Lange B, Van Cauwenberge P, Bachert C. Up-regulation of IL-18 in allergic rhinitis. Allergy. 2002;57:825-30.

19. Yoshizawa Y, Nomaguchi H, Izaki S, Kitamura K. Serum cytokine levels in atopic dermatitis. Clin Exp Dermatol. 2002;27:225-9.
20. Kumano K, Nakao A, Nakajima H, Hayashi F, Kurimoto M, Okamura H, Saito Y, Iwamoto I. Interleukin-18 enhances antigen-induced eosinophil recruitment into the mouse airways. Am J Respir Crit Care Med. 1999;160:873-8.
21. Wild JS, Sigounas A, Sur N, Siddiqui MS, Alam R, Kurimoto M, Sur S. IFN-γ–inducing factor (IL-18) increases allergic sensitization, serum IgE, Th2 cytokines, and airway eosinophilia in a mouse model of allergic asthma. J Immunol. 2000;164:2701-10.
22. Yoshimoto T, Tsutsui H, Tominaga K, Hoshino K, Okamura H, Akira S, Paul WE, Nakanishi K. IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils. Proc Natl Acad Sci USA . 1999;96:13962-6.
23. Busse WW, Lemanske RF. Asthma. N Engl J Med. 2001;344:350-62.
24. Kips JC. Cytokines in asthma. Eur Respir J. 2001;18(Suppl 34):24-33.
25. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med. 1989;320:271-7.
26. Heinzmann A, Gerhold K, Ganter K, Kurz T, Schuchmann L, Keitzer R, Berner R, Deichmann KA. Association study of polymorphisms within interleukin-18 in juvenile idiopathic arthritis and bronchial asthma. Allergy. 2004;59:845-9.
27. Kruse S, Kuehr J, Moseler M, Kopp MV, Kurz T, Deichmann KA, Foster PS, Mattes J. Polymorphisms in the IL 18 gene are associated with specific sensitization to common allergens and allergic rhinitis. J Allergy Clin Immunol. 2003;111:117-22.
28. Studnicka M, Hackl E, Pischinger J, Fangmeyer C, Haschke N, Kühr J, Urbanek R, Neumann M, Frischer T. Traffic-related NO2 and prevalence of asthma and respiratory symptoms in seven year olds. Eur Respir J. 1997;10:2275-8.
29. Sichletidis L, Tsiotsios I, Gavriilidis A, Chloros D, Gioulekas D, Kottakis I, Pataka A. The effects of environmental pollution on the respiratory system of children in Western Macedonia, Greece. J Invest Allergol Clin Immunol. 2005;15:117-23.
30. Wyler D, Braun-Fahrländer C, Künzli N, Schindler C, Ackermann-Liebrich U, Perruchoud AP, Leuenberger P, Wuthrich B. Exposure to motor vehicle traffic and allergic sensitization. Epidemiology. 2000;11:450-6.

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Masayuki Shima

Department of Public Health, Hyogo College of Medicine
1-1 Mukogawa-cho, Nishinomiya, Hyogo 663-8501, Japan
E-mail: shima-m@hyo-med.ac.jp