Acta Medica et Biologica
Vol. 37, No. 1, 19-25, 1989

Correlation Between the Severity of Asthma Due to Candida Albicans and the Titer of Precipitin to *Candida albicans*

Kazuharu TSUKIOKA,[1] Masami NAKAMATA[1] and Shigeru HIRONO[2]

[1]Respiratory Division, National Nishi-Niigata Chest Hospital, [2]Internal Medicine, Shirone Kensei Hospital

Received March 6, 1989

Summary. **In order to elucidate the mechanism involved in *Candida albicans* (Candida)-induced asthma, the correlation between three different antibodies to Candida—the IgE antibody, precipitin and hemagglutinin—and the severity of Candida-induced asthma and the type of response after the bronchial provocation test (BPT) was analyzed. A diagnosis of Candida-induced asthma was made when the BPT was positive only for Candida. In the present study, 21 patients with Candida-induced asthma were investigated. Positive BPT responses consisted of immediate asthmatic responses (IAR), dual asthmatic responses (DAR) and late asthmatic responses (LAR), but only DAR and LAR were observed in the present study. The following results were obtained:**

1) The titer of precipitin increased in accordance with the severity of asthma ($p<0.01$), but the titer of the IgE antibody and hemagglutinin did not show a consistent relation to the severity of asthma.

2) There was no significant correlation between the titer of the IgE antibody, precipitin and hemagglutinin to Candida and the type of response after BPT (DAR and LAR).

These results indicate the possibility that there are cases in which precipitin may play an important role in the development of Candida-induced asthma, especially in sever cases.

INTRODUCTION

We determined allergens in patients with bronchial asthma (referred to as asthma below), designated those cases for which no allergens other than *Candida albicans* (referred to as Candida below) could be confirmed as Candida-induced asthma and studied the mechanism of occurrence of this type of asthma[1-3]. The results revealed that in most cases of Candida-induced asthma, the titer of total IgE was within the normal range and that of specific IgE was negative or low, that late asthmatic response (LAR) or dual asthmatic response (DAR) was common in the bronchial provocation test (BPT), and that these cases showed marked contrast to cases for which allergens other than house dust could not be identified.

It is thought that a mechanism other than the IgE antibody is important in Candida-induced asthma. Since the Candida is continually retained in the body, it is considered to have a sensitizing route different from that of house dust and other inhaled allergens. Consequently, to clarify the mechanism of its occurrence, it is necessary to analyze the relation of humoral antibodies other than the IgE antibody, cellular immunity reactions and other factors. In this study, we measured the IgE antibody, hemagglutinin and precipitin in the serum of patients with Candida-induced asthma and investigated their relation to the severity of asthma and bronchial provocation response. A close relation was found between the severity of asthma and the titer of precipitin. This finding, with importance in clarifying not only the mechanism of occurrence of Candida-induced asthma but also that of the severity of asthma, is reported here.

SUBJECTS AND METHODS

Subjects: A skin test for 45 allergens (Torii Pharmaceutical Co., Ltd.) was performed on asthma patients and a BPT was performed for allergens for which the immediate response was positive. Of the 26 patients who were positive only for candida, 21 who showed DAR or LAR, described later, were selected as subjects. The reason for restricing the subjects to those with DAR or LAR was that hemagglutinin and precipitin are not related to the occurrence of immediate astmatic response (IAR) but are considered to

possibly be related to the occurrence of DAR and LAR. It is possible to obtain a positive response in a BPT using allergens for which the immediate skin response is negative, but this study was not extended to cover these cases.

The 21 Candida-induced asthma patients included 10 males and 11 females whose ages ranged from 14 to 61 and averaged 40.8. According to the severity evaluation criteria of the Japanese Society of Allergology, 6 of the patients were severe, 9 were moderately severe and 6 were midlly severe. DAR was observed in 9 patients and LAR in 12. None of the 21 patients had received desensitization therapy using Candida.

Allergen skin test: 1000-fold dilutions were used for house dust, pollen (12 types), foods (8 types), animal epidermis (7 types) and fibers and other varieties (12 types) and 10,000-fold dilutions were used for fungi (5 types: Candida albicans, Aspergillus fumigatus, Penicillium luteum, Alternaria Kikuchiana, Cladosporium cladosporioides). A 0.02 ml injection of each was injected under the skin and 15 min later the diameters of swelling and redness were measured. If the swelling was wider than 9 mm or the redness wider than 20 mm, the response was judged as positive.

BPT: In patients who had been using a bronchodilator, the study was conducted on a day on which the drug had been withdrawn for at least 12 hours and the patient had not suffered an attack. On the day of the study, the patients practiced so that their vital capacity on effort and forced expiratory volume in one second ($FEV_{1.0}$) became nearly stable. Then it was confirmed that their $FEV_{1.0}$ did not drop by more that 10% within 30 min after inhaling 1 ml of saline solution from a nebulizer for 2 min.

The Candida antigen dilution used for inhalation was made by diluting a freeze-dried powder form of Candida (serum type A, Torii Pharmaceutical Co., Ltd.) in physiological saline by 1000 fold, 500 fold and 100 fold. The administered concentration was increased by one concentration a day begining with the lowest concentration until a positive reaction was obtained. One ml of each concentration was inhaled for 2 min from a nebulizer, and immediately, 10, 20 and 30 min and 1, 2, 3, 5, 7 and 24 h after inhalation, continuous vital capacity on effort and $FEV_{1.0}$ were measured. If the $FEV_{1.0}$ decreased by more that 15% from the pre-inhalation value, the response was considered positive. If the response was observed within 1 h after BPT, it was judged to be an IAR, and if it was seen more than 1 h after the BPT, it was judged to be an LAR. If the $FEV_{1.0}$ decreased again by more that 15% from the pre-inhalation value after the IAR recovered naturally or due to treatment or if the IAR did not recover and continued for more than several hours, it was judged as a DAR.

RAST (radioallergosorbent test): Using the Phadebas® RAST kit, the Candida antibody was measured with Candida antibody coupling paper discs made by Pharmacia Company. A RAST score of 2 or greater was judged positive.

Prausnitz-Küstner (PK) response: In the skin of the forearm of a recipient who showed a negative immediate response to a 10,000-fold Candida dilution, 0.1 ml of serum from the patient was injected to make multiple PK sites, and from 24 to 48 h later 0.02 ml of a 10,000-fold Candida dilution was injected in this area, and the diameters of swelling and redness were measured. Swelling greater than 9 mm or redness greater than 20 mm was judged a positive response. In these results, a positive PK reponse was treared as a RAST score of 2.

Bacteria strain used: Candida albicans A-1, J1012 (serum type: A type, Candida A), was used as the antigen for tanned (sensitized by treatment with tannic acid) red cell agglutination and gel precipitation (Ouchterlony test).

Antigen extraction: Extraction of antigen was performed by the methods of Gorin et al.[4] and Shinoda.[5] That is, after bacteria cultured for 48 h at 30℃ in Sabouraud's agar were collected with deionized water and washed three time, the wet bacteria were combined with a 5-fold volume of deionized water and heated at 100℃ for 1 h. The bacteria were centrifuged at 1308xg for 10 min and obtained as a precipitate. A 5-fold volume of 2% KOH was added to the wet bacteria and they were then extracted under heat for 2 h at 100℃ and neutralized with acetic acid. After centrifuging for 10 min at 1308xg, the clear upper layer was concentrated under reduced pressure at 30℃, combined with a 4-fold volume of methanol and allowed to stand overnight. The resulting precipitate was washed with ethanol and dried with acetone to yield the antigen.

Composition of the antigen used: The chemical composition of the antigen was analyzed by gas chromatography, and the results showed that the component sugars were mannose and glucose, 4.00 mg and 0.55 mg per 10 mg pure antigen, respectively. The micro-Kjeldahl method revealed that the pure antigen contained 1.88 mg of protein per 10 mg.

Tanned cell agglutination reaction: Boyden's method[6] was used to prepare antigen-sensitized red blood cells. That is, to 10 ml of phoshate-buffered saline containing 100 mg of antigen (pH 7.2, PBS

bellow), an equal volume of a 2% PBS suspension of tanned sheep red blood cells was added, and this was incubated for 1 h at 37℃. It was then centrifuged for 10 min at 872xg, and the sensitized blood cells which had precipitated were obtained and washed three times with 1% rabbit serum-added PBS to yield antigen-sensitized red blood cells.

Measurement was done by a microtitration method. A microtitration plate (cooke Company) was used and the blood serum to be examied was diluted with 1% rabbit serum-added PBS to obtain a 0.025 ml series of 2-fold dilutions. Then 0.025 ml of antigen-sensitized red blood cells was added to each, incubated for 2 h at 37℃ and let stand for 10 h at 4°C. The largest serum dilution that showed agglutination of sensitized red blood cells was used to express the titer of red blood cell hemagglutinin.

Gel precipitation response: This was done by Ouchterlony's plate micro method. Torii pharmaceutical's Candida A freeze-dried powder and Candida A powder prepared by the writers were used as the antigen in the wells, and these were diluted with Bernard's buffer (pH 8.6) to give 100-fold, 1,000-fold, 10,000-flod and 100,000-flod dilutions. The patients' undiluted serum was injected in the middle wells. The wells were observed for 1 week, and if a precipitation line appeared between the serum and Torii Pharmaceutical's or our 100 fold antigen dilution, it was judged as (+), and if precipitation lines were observed between the serum and the remaining concentration solutions, they were judged (++), (+++) and (++++), respectively.

Significance test: Significance was determind by U test and rank-difference coefficient and was established at $p<0.05$.

RESULTS

Correlation between asthma severity and the IgE antibody and hemagglutinin (Fig. 1): In cases where the titer of hemagglutinin was negative, 2, 4 or 8, it was expressed with a corresponding log scale of 0, 1, 2 and 3. As a result, no clear relation was observed between asthma severity and the IgE antibody or hamagglutinin.

Correlation between asthma severity and precipitin (Fig. 2): An increase in the precipitin titer was observed with increasing severities of asthma (rank-difference coefficient: $r'=0.971$ ($p<0.01$), $p<0.05$ in U test).

Correlation between BPT-induced asthma (DAR and LAR) and the IgE antibody, hemagglutinin and precipitin (Fig. 3): No definite correlation was observed with any combination.

Correlation between hemagglutinin and precipitin (Fig. 4): A definite relation was observed with a rank-difference coefficient $r'=0.728$ ($p<0.01$).

DISCUSSION

In addition to type I allergic reactions, type III and IV allergic reactions can also be considered to be involved in the occurrence of asthma.[7] Pepys et al. reported that since type III skin reaction in asthmatic patients is closely related to the existence of precipitin,[8,9] it is possible that late airway asthmatic response is caused by type III allergic reaction due to precipitation of the immune complex (IC).[9] Callerame et al.[10] observed hypertrophy of the bronchial basal layer and precipitation of IgG, IgaA, IgM and components of the complement in the basal layer and so suggests that type III allergic reaction is involved in the occurrence of asthma through IC. Kimura et al.[11] reported that peripheral blood basophils in atopic asthma patients with a high serum IgE value reacted strongly to anti-human IgE, but that peripheral blood basophils in patients with a low serum IgE value reacted more strongly to anti-human IgG than anti-human IgE. They suggested that IgG plays a major role in the progress of severe, intractable asthma or in the mechanism of its occurrence.

There are also reports that suggest type III allergic reactions play a role in the occurrence of asthma based on the results of desensitization therapy. When desensitization therapy is performed for Alternaria or Cladosporium, in patients in whom precipitin against these antigens is demonstrated, a general late asthmatic response or serious asthmatic response accompanied by cyanosis is induced several hours later, thus suggesting a relation between percipitin and type III allergic reactions.[12,13]

The mechanism of the occurrence of late bronchial asthmatic response seen in DAR and LAR (isolated LAR) has not yet been clarified. Dolovich et al.[14] showed that late skin response occurred without the presence of IC and was caused by the IgE antibody. In the same way, LAR to BPT occurred in asthma patients in whom precipitin was not found, [15,16] suggesting that the response was induced by the IgE antibody.[16] A secondary condition of type I allergic reactions is inflammation widely assumed to be caused by inflammation-causing proteins, Such as inflammatory factors of anaphylaxis (IF-A)[17] from mast cells and major basic proteins (MBP)[18-20] of eosinophils.

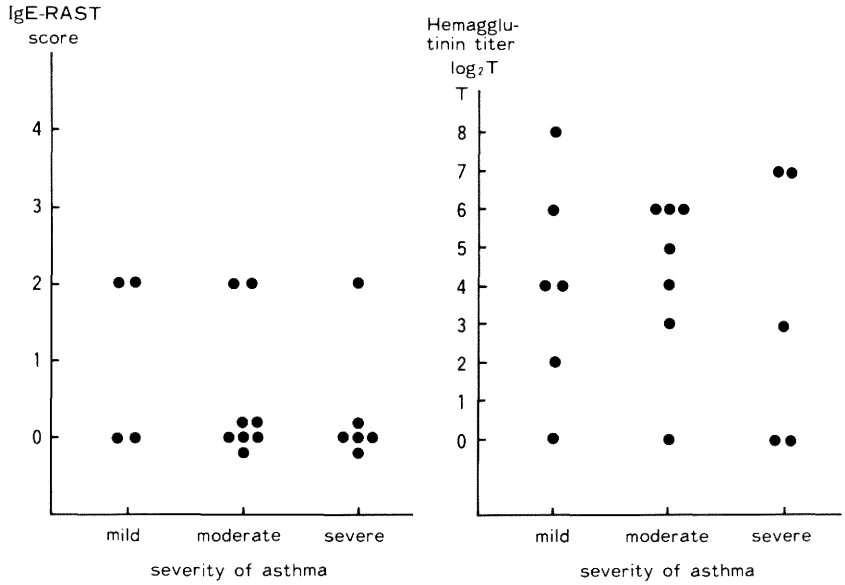

Fig. 1. Correlation between the IgE antibody and hemaglutinin to *Candida albicans* and severity of bronchial asthma induced by *Candida albicans*. Neither the score of IgE-RAST nor the titer of hemagglutinin showed a consistent correlation with the severity of asthma.

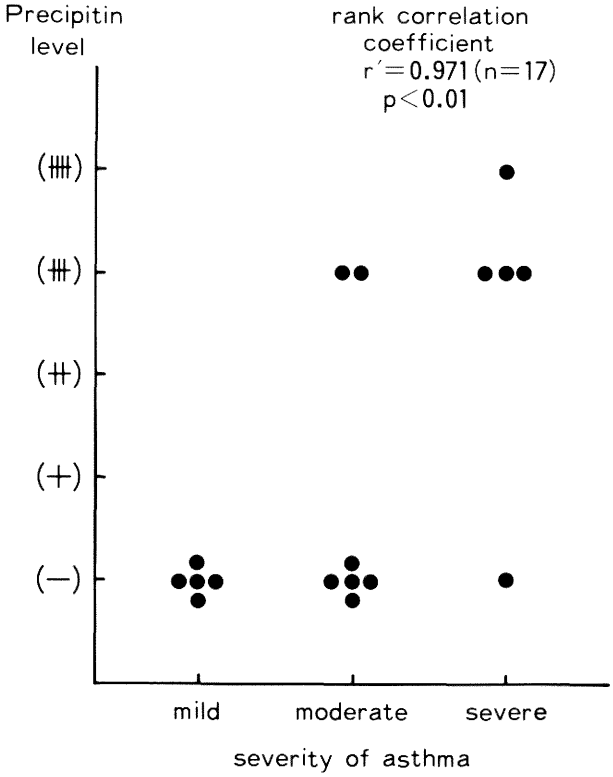

Fig. 2. Correlation between precipitin level to *Candida albicans* and severity of asthma induced by *Candida albicans*. There was a significant correlation between the two ($p<0.01$).

However, there are also opinions that it is caused by type III allergic reaction. Ito et al.[21,22] performed BPT using mite extract in asthma patients positive to mite in RAST or skin tests and reported that LAR (DAR) was clearly observed in a high percentage of cases with high IgG_1 antibody titer before occurrence and cases with high IC levels before occurrence and that it is possible that the IgG (G_1) antibody reacts with the antigen to make IC and the IgG-IC combines with the complement to cause LAR due to type III reaction.

Okada et al.[23] reported that in a BPT using house dust and Candida, the IgE antibody was observed in a high rate in the peripheral blood of patients showing IAR and the IgG antibody in a high rate in patients showing LAR and that the density of immunoglobulin bonding to the surface of peripheral blood basophils in patiens exhibiting LAR to house dust showed a high IgG/IgE antibody count ratio compared to that in patients exhibiting IAR. The same tendency was seen in patients exhibiting LAR to Candida.

In this study of Candida-induced asthma, the increase in asthma severity in patients exhibiting LAR (DAR and LAR) correlated to an increase in the titer of serum precipitin. The main antibody causing agglutination is IgM,[24] and the IgM and IgG antibodies are involved in the precipitin reaction,[25] but the IgG antibody is known to play the principal

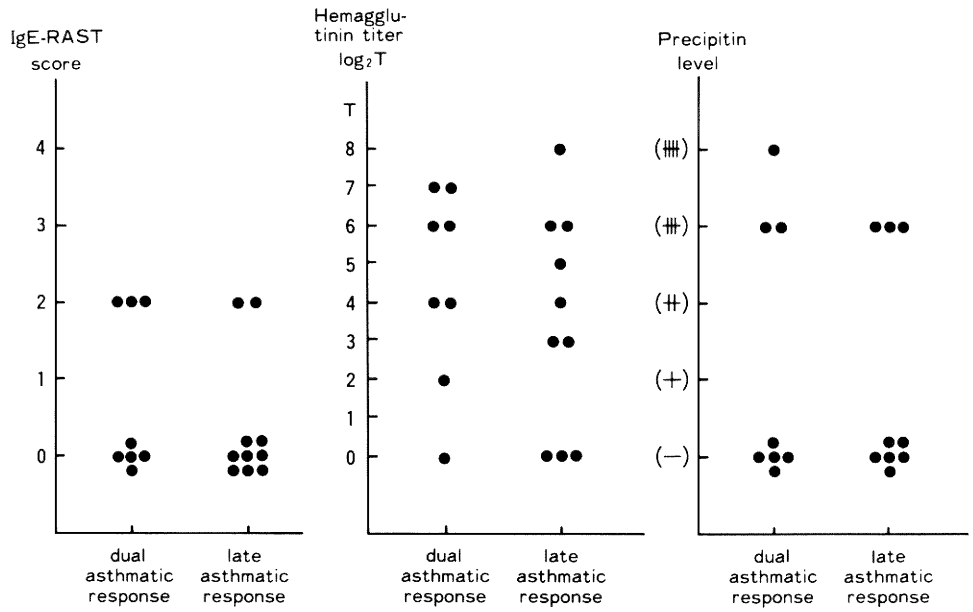

Fig. 3. Correlation between three different antibodies to *Candida albicans*, i.e. the IgE antibody, hemagglutinin and precipitin, and types of bronchial response after the bronchial provocation test using *Candida albicans* extract as the antigen. No consistent correlation was observed.

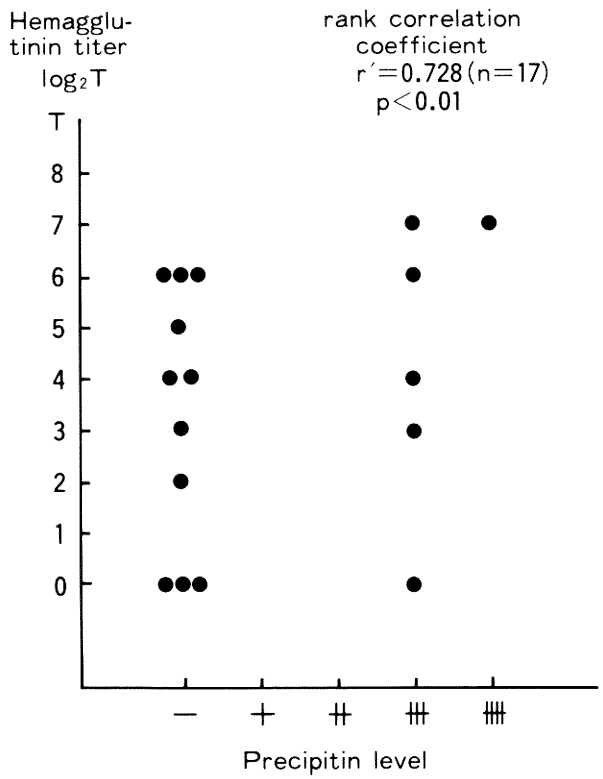

Fig. 4. Relationship between hemagglutinin titer and precipitin level to Candida albicans. Significant correlation was found to exist between them ($p<0.01$).

role.[26] In this study, there were cases in which precipitin was involved in the mechanism of occurrence of Candida-induced asthma, and asthma in these cases seemed to become serious more easily. As suggested by Ito et al.,[21,22] the IgG antibody contained in precipitin probably bonds with the antigen to form IC, and the IgG-IC activates the complement to cause LAR due to a type III response, thus worsening the seriousness of the asthma. However, we have already reported that the IgE antibody readily causes isolated LAR in Candida-induced asthma[3] and that 74% of the isolated LAR seen in BPT of Candida-induced asthma is not caused by the IgE antibody.[27] If one assumes the IgG antibody (precipitin) to Candida travels from inside the blood vessels to local sites without the presence of IAR caused by the IgE antibody, the mechanism is not clear. Therefore, in order to determine which IgG subclass precipitin corresponds to and to deny the possibility that the IAR is a false negative, it is necessary to measure the IgG_4. Type IV response is also considered to play s role in the mechanism of occurrence of Candida-induced asthma,[28] the mechanism of which is likely to be complex.

Acknowledgement. We would like to express our appreciation to Tadashi Hashimoto, M. D., Director of the National Nishi-Niigata Chest Hospital, for his continuous

encouragement and the encouragement and advice received from Masatoshi Niwayama, M. D., Assistant Professor at the Niigata University Health Management Center.

REFERENCES

1) Tsukioka K : Studies on the mechanism developing bronchial asthma due to *Candida albicans*—1. A comparative study of clinical features among Candida-induced asthma and house dust-induced asthma. *Jap J Allergol* **30**: 919-929, 1981. (Japanese with English abstracts)
2) Tsukioka K : Studies on the mechanism developing bronchial asthma due to *Candida albicans*—2. The production of IgE antibody against *Candida albicans* in atopic patients with bronchial asthma. *Jap J Allergol* **31** : 1029-1034, 1982.(Japanese with English abstracts)
3) Tsukioka K : Studies on the mechanism developing bronchial asthma due to *Candida albicans*—3. Relationship between types of response after inhalation challenge with Candida albicans and type I, type III allergy. *Jap J Allergol* **34**: 289-296, 1985. (Japanese with English abstracts)
4) Gorin PAJ and Spencer JFT : Galactomannans of trichosporon fermentans and other yeast : proton magnetic resonance and chemical studies. *Can J Chem* **46** : 2299-2304, 1968.
5) Shinoda T : Relationship between the proton magnetic resonance spectrum and the immunological specificity of polysaccharides from several species of Candida. *Jap J Bacteriol* **27**: 27-34, 1972. (Japanese with English abstracts)
6) Boyden SV : The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera. J Exp Med 93 : 107-120, 1951.
7) Pepys J and Hutchcroft BJ : Bronchial provocation tests in etiologic diagnosis and analysis of asthma. *Amer Rev Respir Dis* **112** : 829-859, 1975.
8) Pepys J Faux JA Longbottom JL McCarthy DS and Hargreave FE: Candida albicans precipitins in respiratory disease in man. *J Allergy* **41** : 305-318, 1968.
9) Pepys J Turner-Warwick M Dawson PL and Hinson KFW : Arthus (Type III) skin test reactions in man. Clinical and immunopathological features. In "Excerpta Medica Foundation, No. 162" (Ed. by Rose, B., Richter, M., Sehon, A. and Frankland, A. W.), 1968, pp. 221-235, 1968.
10) Callerame ML Condemi JJ Bohrod MG and Vaughan JH : Immunological reactions of bronchial tissues in asthma. *N Engl J Med* **284** : 459-464, 1971.
11) Kimura I Tanizaki Y Takahashi K Ueda N et al : Reactivity of basophils to immunoglobulin in bronchial asthma—Feasibility of classifying asthma based on reactivity to anti-human IgG. *Jap J Allergol* **25** : 70-75, 1976.(Japanese)
12) Busse WW Storms WW Flaherty DK Crandall M and Reed CE : Alternaria IgG precipitins and adverse reactions. *J Allergy Clin Immunol* **57** : 367-372, 1976.
13) Kaad PH and Φstergaard PAa : The hazard of mould hyposensitization in children with asthma. *Clin Allergy* **12** : 317-320, 1982.
14) Dolovich J Hargreave FE Chalmers R Shier KJ Gauldie J and Bienenstock J : Late cutaneous allergic responses in isolated IgE-dependent reactions. *J Allergy Clin Immunol* **52** : 38-46, 1973.
15) Booij-Noord H Orie NGM and de Vries K : Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. *J Allergy Clin Immunol* **48** : 344-354, 1971.
16) Robertson DG Kerigan AT Hargreave FE Chalmers R and Dolovich J : Late asthmatic responses induced by ragweed pollen allergen. *J Allergy Clin Immunol* **54** : 244-254, 1974.
17) Oertel HL and Kaliner M : The biologic activity of mast cell granules—III. Purification of inflammatory factors of anaphylaxis (IF-A) responsible for causing late-phase reactions. *J Immunol* **127** : 1398-1402, 1981.
18) Gleich GJ Loegering DA and Maldonado JE : Identification of a major basic protein in guinea pig eosinophil granules. *J Exp Med* **137** : 1459-1471, 1973.
19) Gleich GJ Frigas E Leogering DA Wassom DL and Steinmuller D : Cytotoxic properties of the eosinophil major basic protein. *J Immunol* **123**: 2925-2927, 1979.
20) Frigas E Leogering DA and Gleich GJ : Cytotoxic effects of the guinea pig eosinophil major basic protein on tracheal epithelium. *Lab Invest* **42** : 35-43, 1980.
21) Ito K Kudo K Okudaira H Yoshinoya S et al : Changes of serum proteins including antibodies and of chemical mediators in the time course of dual asthmatic response. *Jap J Thorac Dis* **24**: 1113-1122, 1986. (Japanese with English abstracts)
22) Ito K Kudo K Okudaira H Yoshinoya S et al : IgG_1 antibodies to house dust mite (Dermatophagoides farinae) and late asthmatic response. *Int Arch Allergy Appl Immunol* **81** : 69-74, 1986.
23) Okada C Takahashi K Soda R Matsuoka T et al : Studies on immunoglobulin molecules bound to basophils in late asthmatic response. *Jap J Allergol* **37** : 5-11, 1988. (Japanese with English abstracts)
24) Kishimoto T and Onoue K : Agglutinating activities of pepsin fragments and subunits of IgM antibodies. *J Immunol* **106** : 536-544, 1971.
25) Taschdjian CL Kozinn PJ and Caroline, L : Immune studies in candidiasis—III. Precipitating antibodies in systemic candidiasis. *Sabouraudia,* **3** : 312-320, 1964.

26) Kawai T Salvaggio J Arquembourg P and Marsh D : Precipitating antibodies against organic dust antigens in human sera by counterimmuno-electrophoresis. *Chest* **64** : 420-426, 1973.
27) Tsukioka K Nakamata M and Hirono S : Studies on the machanism developing bronchial asthma due to Candida albicans—5. A comparative study of mechanism producing bronchial asthma among Candida, other moulds and house dust-induced asthma. *Jap J Allergol* **36** : 1047-1053, 1987. (Japanese with English abstracts)
28) Miyagawa H Namba K Shiraishi T Nabe M et al : Studies on cell-mediated hypersensitivity in pathogenesis of intractable asthma. *Jap J Allergol* **37** : 12-18, 1988. (Japanese with English abstracts)