

Development of serum *Dermatophagoides farinae*-, ovalbumin- and lactalbumin-specific IgG, IgG₁, IgG₄, IgA and IgM in children with bronchial asthma/allergic rhinitis or atopic dermatitis

H. OKAHATA, Y. NISHI*, N. MIZOGUCHI, C. YUMIBA, H. FUJII† and K. UEDA‡

Department of Pediatrics, Ohtake National Hospital, Ohtake, *Department of Pediatrics, Hiroshima Red Cross Hospital, Naka-ku, †Department of Pediatrics, Funairi Hiroshima City Hospital, Naka-ku, ‡Department of Pediatrics, Hiroshima University School of Medicine, Minami-ku, Hiroshima, Japan

Summary

Dermatophagoides farinae-, ovalbumin- and lactalbumin-specific IgG, IgG₁, IgG₄, IgA and IgM were evaluated in 161 healthy children [Group 1], 84 children with bronchial asthma and/or allergic rhinitis but without atopic dermatitis [Group 2], and 54 children with atopic dermatitis but without bronchial asthma and allergic rhinitis [Group 3]. We also studied *D. farinae*-, egg-white-, and milk-specific IgE of children with allergic diseases. *D. farinae*-specific IgG, IgG₁, IgG₄ and IgA in Groups 2 and 3 increased until 5 years of age and thereafter they remained constant. After 2 years of age, *D. farinae*-specific IgG, IgG₁, IgG₄ and IgA in Group 2 were higher than those in Groups 1 and 3. Ovalbumin- and lactalbumin-specific IgG, IgG₁, IgG₄ and IgA in Groups 2 and 3 increased until 1 year of age and thereafter decreased. Until 1 year of age, ovalbumin- and lactalbumin-specific IgG, IgG₁ and IgG₄ in Groups 3 were higher than those in Groups 1 and 2. *D. farinae*-, ovalbumin- and lactalbumin-specific IgM were constant in all ages of all groups. These results suggest that atopic dermatitis in young children is related to food-specific immunoglobulins and that respiratory allergic diseases in older children is related to *D. farinae*-specific immunoglobulins.

Clinical and Experimental Allergy, Vol. 20, pp. 39–44. Submitted 5 April 1989; revised 20 June 1989; accepted 20 July 1989.

Introduction

In allergic diseases, IgE is regarded as the most important immunoglobulin, but other immunoglobulins such as IgG and IgG₄ have recently been reported to play an important role in allergic diseases [1–4]. Few studies have been made on the relationship between these immunoglobulins and respiratory and skin allergic diseases. In this study, the concentrations of *Dermatophagoides farinae* (mite-, ovalbumin- and lactalbumin-specific IgG, IgG₁, IgG₄, IgA and IgM were examined in children with bronchial asthma/allergic rhinitis or atopic dermatitis

and in healthy children, and the relationship between allergen specific immunoglobulins and allergic diseases was analysed.

Materials and methods

Study population

Blood was drawn from 161 healthy children without allergic diseases aged 6 months to 15 yr (Group 1), 84 out-patients with bronchial asthma and/or allergic rhinitis but without atopic dermatitis aged 1–15 yr (Group 2), and 54 out-patients with atopic dermatitis without bronchial asthma and allergic rhinitis aged 6 months to 5 yr and having no evident immediate-type allergic reactions to food allergens (Group 3). The drawn blood was kept at room temperature for 2–3 hr and thereafter sera were

Correspondence: Dr Hiroyasu Okahata, Department of Pediatrics, Ohtake National Hospital, 4-1-1 Kuba, Ohtake, Hiroshima 739-06, Japan.

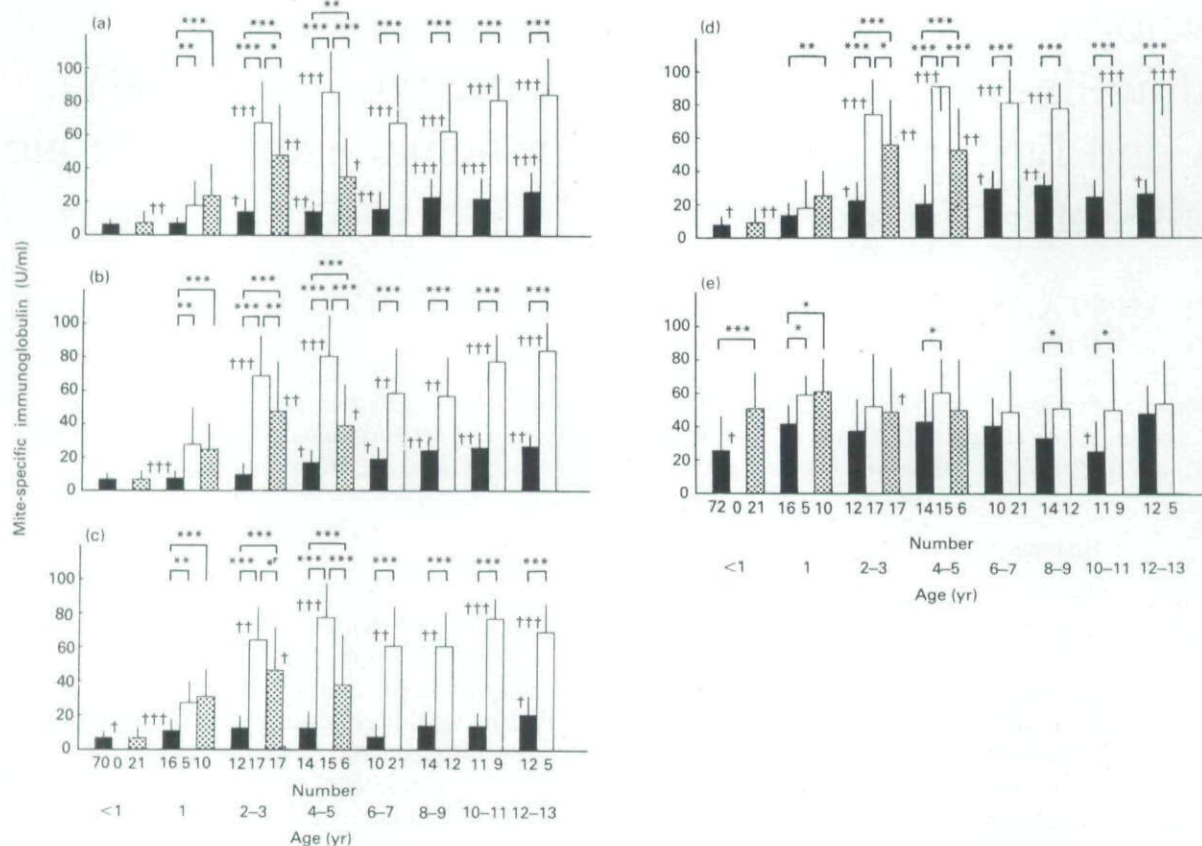


Fig. 1. Mite-specific immunoglobulins; (a) IgG, (b) IgG₁, (c) IgG₄, (d) IgA, (e) IgM; in allergic and non-allergic children. (■) Children without allergic diseases (Group 1); (□) children with bronchial asthma and/or allergic rhinitis but without atopic dermatitis (Group 2); (▨) children with atopic dermatitis but without respiratory allergic diseases (Group 3). * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$. † $P < 0.05$; †† $P < 0.02$; ††† $P < 0.01$; these data were compared with the data of the same group at 1 year of age.

separated and stored at -20°C until allergen-specific immunoglobulin assays were performed. Children with allergic diseases received no elimination diet or hypo-sensitization therapy. Informed consent was obtained from each subject or his or her parents.

Determination of antibodies to mite, ovalbumin and lactalbumin

Allergen-specific IgG, IgG₁, IgG₄, IgA and IgM antibodies in sera were measured by enzyme-linked immunosorbent assays (ELISA) according to the methods of Engvall *et al.* [5] and Kitani *et al.* [6]. Crude extracts of mite were kindly supplied by Torii & Co. Ltd, Tokyo, Japan), ovalbumin (Sigma Chemical Co., St Louis, U.S.A.) and lactalbumin (Sigma Chemical Co.) were each dissolved in 0.3 M carbonate buffer (pH 9). One hundred microlitres of a 10 $\mu\text{g}/\text{ml}$ solution of the mite, ovalbumin and lactalbumin extracts were placed in pairs of wells with two left free from antigen in each 96-well polystyrene

microtitre plate (Becton Dickinson Labware, Oxnard, U.S.A.) and incubated at 4°C overnight. The allergen solution was then aspirated and the wells were washed three times with 0.067 M phosphate-buffered saline (PBS) pH 7.4 containing 0.05% Tween 20 (Katayama Chemical Co., Osaka, Japan) (PBS-T). After 100 μl of 1% bovine serum albumin (Sigma Chemical Co.) (BSA) in PBS (BSA-PBS) were placed in each well and incubated at 4°C overnight, the plate was washed three times with PBS-T. This procedure was applied to reduce non-specific reaction. One hundred microlitres of each serum diluted appropriately were placed in the well. Usually sera diluted to 1:1000 with PBS-BSA were used for measurement of IgG, IgG₁, IgG₄, IgA and IgM antibodies. After incubation at 4°C overnight, the sera were aspirated and the plates were washed three times as described above. Then, 100 μl of anti-human IgG (Cappel Laboratories, Cochranville, PA, #26135 diluted 1000 times with PBS-BSA), IgG₁ (Bio-Yeda Laboratories, Kiryat Weizmann, Israel, #M895; diluted 1000 times with PBS-BSA), IgG₄ (Bio-

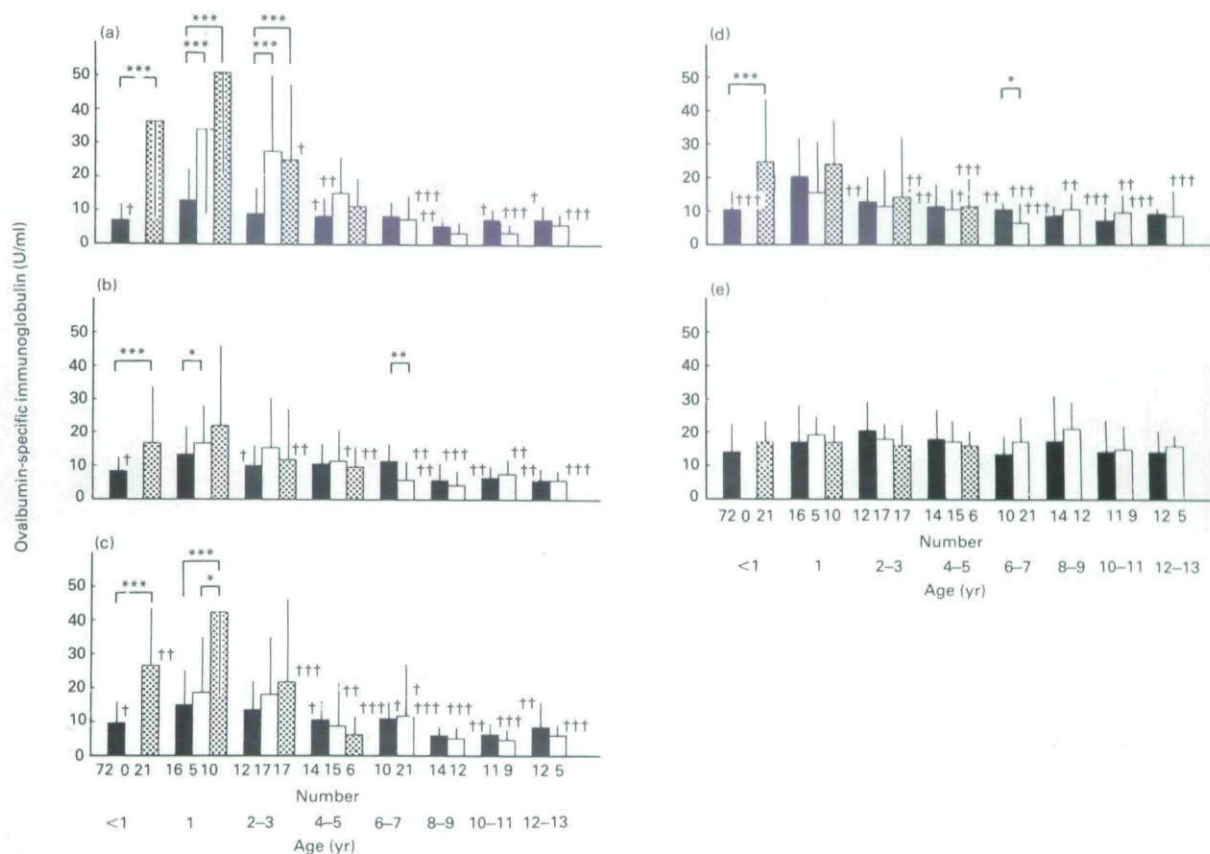


Fig. 2. Ovalbumin-specific immunoglobulins; (a) IgG, (b) IgG₁, (c) IgG₄, (d) IgA, (e) IgM; in allergic and non-allergic children. (■) Children without allergic diseases (Group 1); (□) children with bronchial asthma and/or allergic rhinitis but without atopic dermatitis (Group 2); (▨) children with atopic dermatitis but without respiratory allergic diseases (Group 3). * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$. † $P < 0.05$; †† $P < 0.02$; ††† $P < 0.01$; these data were compared with the data of the same group at 1 year of age.

Yeda Laboratories; #RO28; diluted 1000 times with PBS-BSA), IgA (Yamasa Laboratory, Noda, Chiba, Japan #121; diluted 10 000 times with PBS-BSA) or IgM (Cappel Laboratories, #24022; diluted 1000 times with PBS-BSA) monoclonal antibodies were placed in each well and incubated at 4°C overnight. After incubation, these monoclonal antibodies were aspirated and the wells washed three times. Following these procedures, 100 µl of peroxidase-conjugated anti-mouse IgG (Bio-Yeda Laboratories, #E777; diluted 500 times with PBS-BSA) were placed in each well and incubated at 4°C overnight. After incubation, the anti-mouse IgG was aspirated and the wells were washed five times. In each well of the microtitre plate, 100 µl of 2,2'-amino-di-(3-ethylbenziazoline sulphonate) (ABTS) were added. After incubation for 1 hr at room temperature, the enzyme reaction in each well was stopped by adding 100 µl of 0.01% sodium azide in 0.1 M citric acid. The absorbance was measured in a spectrophotometer (Toso, Tokyo, Japan) at 405 nm. The absorbance data were calibrated by dilution of a human

reference serum with a high titre of allergen-specific immunoglobulin, arbitrarily ascribed 100 U/ml of undiluted serum.

Allergen-specific IgE of children belonging to Groups 2 and 3 was measured by IgE RAST (Pharmacia Fine Chemicals Co., Uppsala, Sweden).

Statistics

Data are presented as mean $\pm$ s.d. U/ml and all comparisons between the groups were made by non-parametric Mann-Whitney *U*-test.

Results

Mite

Figure 1 shows the results of mite-specific antibody levels in three groups of children. In all three groups, mite-specific IgG, IgG₁, IgG₄ and IgA increased after 1 year of age and there was an evident difference among the groups

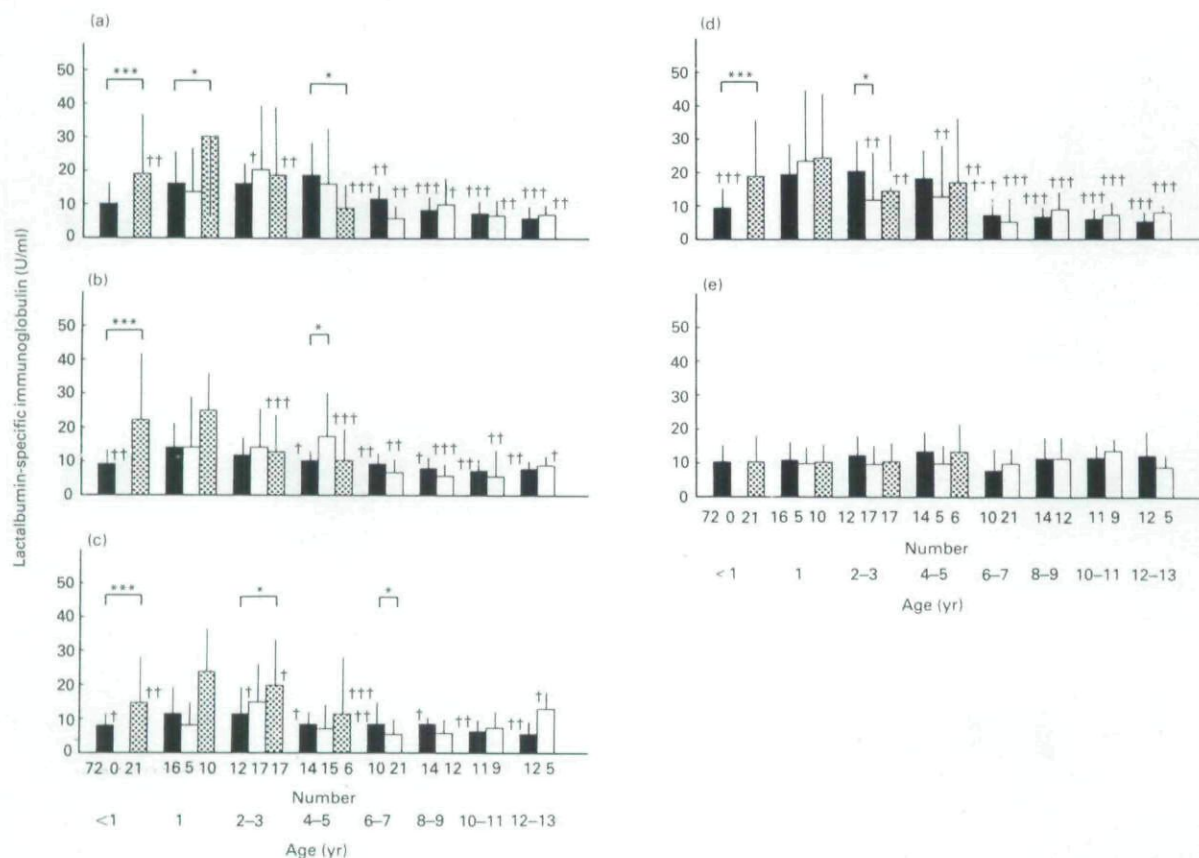


Fig. 3. Lactalbumin-specific immunoglobulins; (a) IgG, (b) IgG₁, (c) IgG₄, (d) IgA, (e) IgM; in allergic and non-allergic children. (■) Children without allergic diseases (Group 1); (□) children with bronchial asthma and/or allergic rhinitis but without atopic dermatitis (Group 2); (▨) children with atopic dermatitis but without respiratory allergic diseases (Group 3). * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$. † $P < 0.05$; †† $P < 0.02$; ††† $P < 0.01$; these data were compared with the data of the same group at 1 year of age.

after 2 years of age, whereas mite-specific IgM showed no increments until 15 years of age. Mite-specific IgG, IgG₁, IgG₄ and IgA in Groups 2 and 3 were higher than those in Group 1 each year after 1 year of age, and those in Group 2 were higher than those in Group 3 after 2 years of age.

Ovalbumin

Ovalbumin-specific IgG, IgG₁, IgG₄ and IgA in all groups increased until 1 year of age and decreased thereafter (Fig. 2). Ovalbumin-specific IgG, IgG₁, IgG₄ and IgA in Group 3 were higher than those in Group 1 until 2 years of age. Ovalbumin-specific IgG in Groups 2 and 3 were higher than those in Group 1 aged 1–3 yr, and ovalbumin-specific IgG₄ in Groups 2 and 3 were higher than those in Group 1 at 1 year of age. There was no apparent difference in IgM among the three groups.

Lactalbumin

Lactalbumin-specific IgG, IgG₁, IgG₄ and IgA in all groups increased until 1 year of age and then decreased

(Fig. 3). The antibodies in Group 3 were higher than those in Group 1 from 6 months to 1 year of age and lactalbumin-specific IgG, IgG₁ and IgG₄ in Group 3 were higher than those in Group 1 at 1 year of age. Lactalbumin-specific IgM in all groups from 6 months to 15 years of age did not show any statistical differences.

IgE study

Mite-, egg- white- and milk-specific IgE levels showed almost the same tendency as mite-specific IgG, IgG₁, IgG₄ and IgA (Fig. 4).

Discussion

This study showed that mite-specific IgG, IgG₁, IgG₄, IgA and IgE increased until 5 years of age and thereafter remained constant, whereas mite-specific IgM levels remained constant in all age groups. Ovalbumin- and lactalbumin-specific IgG, IgG₁, IgG₄ and IgA in all

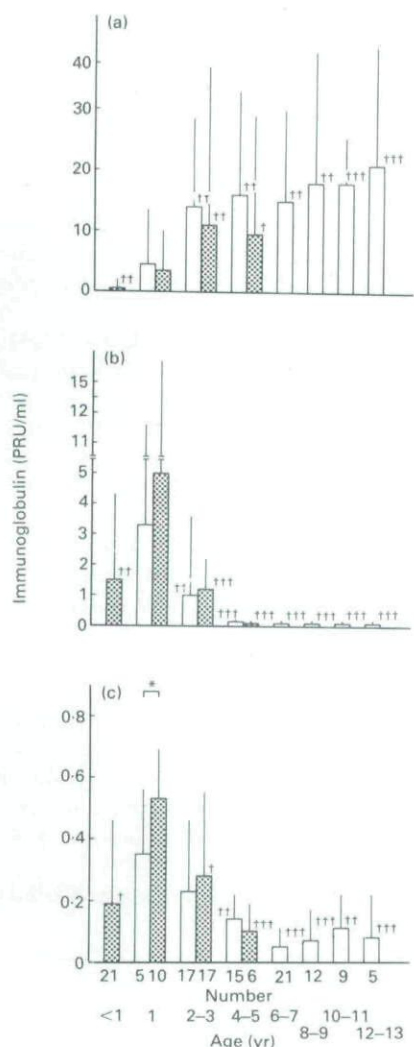


Fig. 4. (a) Mite-, (b) egg-white- and (c) milk-specific IgE in children with allergic diseases. (□) Children with bronchial asthma and/or allergic rhinitis but without atopic dermatitis (Group 2); (▨) children with atopic dermatitis but without respiratory allergic diseases (Group 3). * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$. † $P < 0.05$; †† $P < 0.02$; ††† $P < 0.01$; these data were compared with the data of the same group at 1 year of age.

groups were found to be high at 1 year of age but decreased thereafter. These antibody levels of Groups 2 and 3 (especially Group 3) were higher than those in Group 1.

Recently many investigators have demonstrated a relationship between allergic disorders and allergen specific IgG, IgG₁ and IgG₄, but few studies have been made on the relationship among mite and food allergen-specific immunoglobulins (IgG, IgG₁, IgG₄, IgA and IgM) in patients with respiratory allergic disorders alone and in patients with atopic dermatitis alone with increase in age [1-4,7,8].

Dannaeus [9] studied the relationship between diet and specific antibodies (IgA, IgM) in asthmatic infants and observed that, in infants, the permeability of the gut to allergens was high and when infants were massively exposed to food antigens, antigens reached antibody-forming cells and a humoral immune response developed to ingested food. He also found that in addition to IgG-, IgA- and IgM-food antibodies, atopic children began to develop IgE antibodies to food. Rawle *et al.* [10] reported that asthma and hay fever patients with spontaneous improvement showed a progressive fall in both IgG and IgE antibodies to dust mite antigen and pollen allergen. Their results suggest that IgG antibody to antigen may serve not only as a blocking antibody but may have also atopic effects. In this study we observed that there were few atopic dermatitis patients without respiratory allergic disorders after 5 years of age and that from 4 to 5 years of age ovalbumin- and lactalbumin-specific IgG, IgG₁, IgG₄ and IgA decreased to the values of healthy controls. These results suggest that food-specific immunoglobulins play almost the same role in atopic dermatitis patients as in Rawle *et al.*'s study, and that the relief of atopic dermatitis may be related to the permeability of the gut to food allergens and these allergen-specific immunoglobulin values.

While there are many inhalant allergens, house dust mite is regarded as a common inhalant allergen particularly in patients with perennial respiratory allergy [11]. Sarsfield [12] showed that mite sensitivity develops early in life and that by the age of 2 yr half of an unselected group of asthmatics had a positive skin reaction to *Dermatophagoides*. Mukoyama *et al.* [13] demonstrated that IgG antibodies to mite were elevated in asthma and atopic dermatitis children and that IgG antibodies seemed to be correlated to IgE antibodies. These studies are partially in accordance with our study that mite-specific IgG, IgG₁, IgG₄, IgA and IgE increase, especially in asthma patients, as age increases. Furthermore, these studies and our results imply that the factors controlling the incidence of inhalant allergy control IgG and IgA antibodies' production as well as IgE antibody.

Kitani *et al.* [6], during studies of the IgG, IgA and IgM antibodies to mite in sera and sputa from asthmatic patients, concluded that IgA antibodies to mite in sputa were significantly higher in mite-sensitive patients than in normal controls or mite-insensitive asthmatic patients; whereas IgG and IgA antibodies in sera were significantly higher in mite-sensitive patients than in the other two groups, and that there were no significant differences in serum or sputum IgM antibodies among the three groups. They also concluded that IgA antibodies in bronchial secretions may play a protective role in asthma when allergens enter into the bronchial tree. Their studies were

partially in agreement with our study in which the serum mite-specific IgG, IgG₁, IgG₄ and IgA in respiratory allergy (and atopic dermatitis) patients with high mite-specific IgE were higher than those in normal controls with low mite-specific IgE. Yamaguchi *et al.* [14] showed that secretory IgA levels were lower in asthmatic children with food allergy than in asthmatic children without food allergy or in healthy children, and that secretory IgA may play an important role in food allergy. These studies suggest that IgA antibodies have an important influence on allergic disorders. The results of our study did not demonstrate IgA specificity in allergic disorder children but showed that mite-, ovalbumin- and lactalbumin-specific (food allergen specific) IgG, IgG₁, IgG₄ and IgA in children with respiratory allergy and atopic dermatitis were higher than those in control children.

In conclusion, our results showed that allergen-specific IgG, IgG₁, IgG₄ and IgA changed like allergen-specific IgE, and that older children with respiratory allergy disease had high mite-specific immunoglobulins, and younger children with atopic dermatitis had high food allergen-specific immunoglobulins. These findings suggest that these allergens affect the formation of allergen-specific IgG, IgG₁, IgG₄ and IgA. It was also demonstrated that allergen-specific IgM did not change by age or by disease.

References

- Ishizaka K, Ishizaka T, Hornbrook M. Physicochemical properties of reaginic antibody IV: presence of unique immunoglobulin as a carrier of reaginic activity. *J Immunol* 1966; 97:75-85.
- Ishizaka K, Ishizaka T. Identification of E antibodies as a carrier of reaginic activity. *J Immunol* 1967; 99:1187-98.
- Lichtenstein LM, Norman PS, Winkenwerder WL. A single year of immuno-therapy for ragweed hay fever. *Ann Intern Med* 1971; 75:663-71.
- Aalberse RC, Van der Gaas R, Van Leeuwen J. Serologic aspects of IgG₄ antibodies. I. Prolonged immunization results in an IgG₄-restricted response. *J Immunol* 1983; 130:722-6.
- Engvall E, Perlmann P. Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labeled antiimmunoglobulin in antigen-coated tubes. *J Immunol* 1972; 109:129-35.
- Kitani S, Ito K, Miyamoto T. IgG, IgA and IgM antibodies to mite in sera and sputa from asthmatic patients. *Ann Allergy* 1985; 55:612-20.
- Chapmann MD, Platts-Mills TAE. Measurement of IgG, IgA and IgE antibodies to *Dermatophagoides pteronyssinus* by antigen-binding assay, using a partially purified fraction of mite extract (F4P1). *Clin Exp Immunol* 1978; 34:126-36.
- Sakamoto Y, Nakagawa T, Ito K, Miyamoto T. Solid-phase radioimmunoassay for the measurement of IgG antibodies specific for the house dust mite, *Dermatophagoides farinae*. *Ann Allergy* 1984; 52:303-8.
- Dannaeus A. Diet and asthma in infancy and childhood. *Eur J Respir Dis* 1984; 65(suppl):165-7.
- Rawle FC, Burr ML, Platts-Mills TAE. Long-term falls in antibodies to dust mite and pollen allergens in patients with asthma or hay fever. *Clin Allergy* 1983; 13:409-17.
- Bierman CW, Pearlman DS. Allergic diseases of infancy, childhood and adolescence. Philadelphia: W.B. Saunders Co., 1980.
- Sarsfield JK. Role of house-dust mites in childhood asthma. *Arch Dis Child* 1974; 49:711-14.
- Mukoyama T, Ichikawa K, Baba M, Nakagawa T, Miyamoto T. Specific antibodies of IgE, IgG and IgG₄ to *Dermatophagoides pteronyssinus* and ovalbumin in atopic children. *Jpn J Allergol* 1986; 35:166-70.
- Yamaguchi K, Mukoyama T, Baba M. Secretory IgA in saliva of asthmatic children. *Jpn J Allergol* 1985; 34:234-8.

Copyright of Clinical & Experimental Allergy is the property of Blackwell Publishing Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.