

A COMPARISON BETWEEN IgE AND IgG4 AS MARKERS OF ALLERGY IN CHILDREN: AN EXPERIMENTAL TRIAL IN A MODEL OF NATURAL ANTIGEN AVOIDANCE

G.L. PIACENTINI¹, S. GUERRESI¹, A. KANTAR², L. LUBRANO¹, F. OLIVIERI¹,
A.L. BONER¹ and D.G. PERONI¹

¹*Pediatric Section, Department of Life and Reproduction Sciences, University of Verona, Verona;*

²*Istituto Pio XII, Misurina (BL), Italy*

Received May 17, 2011 – Accepted October 21, 2011

IgG4 have been hypothesized to act as blocking antibodies capable of preventing IgE-mediated effector cell triggering. This study aims to evaluate the changes in IgG4 in children during a period of natural antigen avoidance. Serum IgE and IgG4 were evaluated in a group of asthmatic children, aged between 7 and 17 years, admitted to the residential house Istituto Pio XII (Misurina, BL, Italy), located at 1,756 m, in a natural model of antigen avoidance. All the patients were skin prick test positive to at least two of the following allergens: *Dermatophagoides pteronissynus*, *Dermatophagoides farinae*, cat epithelium, timothy grass pollen and Parietaria pollen. During the 180 days of hospitalization, serum specific IgE and IgG4 were measured six times. A significant decrease ($p \leq 0.05$) in serum specific IgE to house dust mite and pollen allergens was observed; by contrast, no significant variations were shown by IgG4 and IgG4/IgE ratio. No significant relationship was found between serum specific IgE, IgG4 and IgG4/IgE ratio variations and the re-exposure to house dust mite allergens during the Christmas holidays. A positive correlation between specific IgE and specific IgG4 was observed at each considered time (T0: $r=0.57$, $p=0.08$; T1: $r=0.85$, $p=0.001$; T3: $r=0.76$, $p=0.01$). The positive correlation between specific IgE and specific IgG4, enduring throughout the entire time of study, suggests a relationship between these classes of immunoglobulins.

Exposure to allergens induces an immunologic response that involves both serum allergen specific IgE and IgG4 (1) which have been suggested to represent soluble markers of sensitization and allergic disease (2).

Both IgG4 and IgE are regulated by Th2 lymphoid cells (3-4). IgG4, in particular, have been hypothesized to act as blocking antibodies capable of preventing IgE-mediated effector cell triggering (5), possibly by an allergen-blocking effect at the

level of mast cells and/or antigen-presenting cells (6). Furthermore, IgG4 antibodies have been shown to be expressed after prolonged immunization and they have been hypothesized to be involved in the development of tolerance (7-10).

A number of studies performed in an Alpine sanatoria (11-17) have demonstrated that, in asthmatic children, staying at a high altitude is associated with a global improvement of the disease, including clinical respiratory symptoms, lung

Key words: antigen avoidance, asthma, IgE, IgG4

Mailing address: Diego G. Peroni, MD
Paediatric Department,
Department of Life and Reproduction Sciences,
University of Verona, Policlinico G.B. Rossi,
P.le L.A. Scuro, 37134 Verona, Italy
Fax: ++39 045 8124790
e-mail: diego.peroni@univr.it

0394-6320 (2011)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

function and bronchial hyperreactivity, a reduction in markers of allergic inflammation and in specific IgE levels (11-17).

To the best of our knowledge, to date no study has investigated changes in allergen-specific IgG4 after protracted avoidance. The present study was carried out to evaluate the variations of serum specific IgG4 and IgE to specific aero-allergens, including *Dermatophagoides pteronyssinus* (d1), *Dermatophagoides farinae* (d2), *Phleum pratense* (g6), *Parietaria judaica* (w21) and cat epithelium (e1) in a group of asthmatic children during a long period of avoidance in an Alpine environment.

MATERIALS AND METHODS

Subjects and experimental design

Children with allergic asthma, guests of the residential house Istituto Pio XII (Misurina, Italian Dolomites, 1,756 m above sea level) from September 2007 to March 2008, were enrolled in this longitudinal study. During the time of the study, they returned home, at sea level, for 10-12 days during the Christmas holidays (end of December-beginning of January).

Routine analysis of dust collected from the children's mattresses in the residential house always failed to show significant concentrations of mites and cat and the study was performed out of season for pollen exposure. Therefore, during their stay in the residential house the children can be considered to undergo a natural period of avoidance to the above referred allergens, whereas during the holiday period they are naturally re-exposed to allergens found in their domestic environments (13-15).

Serum samplings were performed at four different timepoints, between September and March. The first determination was made at the time of arrival in Misurina (T0), the further samplings were performed after 96 (T1), 116 (T2) and 180 (T3) days. The experimental design is summarized in Fig. 1. The study protocol was approved by the Istituto Pio XII ethics committee and both children and parents gave their consent to participate.

Skin prick test

The tests were performed at the time of admission to the residential house Pio XII according to standard methods with allergens, histamine-positive and negative controls purchased from Lofarma (Italy). The skin prick reaction was read at 15 minutes and considered positive if the wheal size induced by the allergen extract was at least 50% of the one elicited by the positive control (18). All patients underwent allergen testing for dust mites, *Dermatophagoides pteronyssinus* (d1),

Dermatophagoides farinae (d2), *Phleum pratense* (g6), *Parietaria judaica* (w21) and cat epithelium (e1).

Lung function test and FeNO

Evaluations of forced expiratory volume in one second (FEV1), forced expiratory volume in one second to forced vital capacity ratio (FEV1/FVC), forced expiratory flow between 50% and 75% FVC (FEF 25-75) and exhaled nitric oxide (FeNO) were performed at the time of admission to the residential house in September, in December after 3 months of stay, in January after 10-12 days at home, exposed to allergens, and in March after 6 months of stay. Spirometries were performed by a Vitalograph Compact spirometer (Vitalograph Ltd., Buckingham, UK) (19). FeNO was evaluated with a chemiluminescence analyser (Logan LR 2149; Logan, Rochester, Kent, UK) as formerly described (20).

Serum specific IgE and IgG4

Antibody titers were determined via the ImmunoCAP 100 assay (Phadia AB, Uppsala, Sweden) as described for IgE (21) and IgG4 (22) (detection limits 0.35 kU/L and 0.15 mg/L, respectively). A zero control was included in the ImmunoCAP assay to extend the limit of detection to 0.1 kU/L (23-24), because of the recent suggestions of a potential usefulness of data on IgE levels below the standard clinical cut-off 0.35 kU/L in children (25).

Statistical analysis

Comparisons between times of the exposure-avoidance study were planned a priori. Student's *t*-test was used for analyzing serum specific immunoglobulin variations between T0 and T1, between T0 and T3, and between T1 and T2 (Fig. 1). We considered statistically significant a *p*-value 0.05.

To investigate possible correlations between IgE and IgG4 and between these antibodies and respiratory parameters we used simple regression test, considering statistically significant a *p*-value ≤ 0.1 .

RESULTS

Twelve children with asthma aged between 7 and 17 years (mean=13 years) were enrolled in the study. They were skin prick test positive to at least two of the following allergens: *D. Pteronyssinus*, *D. Farinae*, *Phleum pratense*, *Parietaria judaica* and cat epithelium (Table I).

Specific IgE, IgG4 and IgG4/IgE serum kinetics

Specific d1, d2, g6, w21 and e1 IgE showed a decrease in all the children during the time of the

Table I. Clinical characteristics of children detected at admission: age, gender, lung function, FeNO, skin prick test positivity and asthma severity.

N°	Sex	Age	Fev1%	Fev1/Fvc%	FeNO ppb	Skin Prick Test +	Asthma Severity
1	M	16	101	93	45	d1-d2-g6-e1	mild
2	M	15	80	96	22,1	d1-d2-e1	mild
3	M	13	99	93	11,5	d1-d2-g6-e1	mild
4	F	12	87	101	28,5	d1-d2-g6-e1	mild
5	M	15	75	84	12,1	d1-d2-g6	moderate
6	M	17	73	77	22,1	d1-d2-g6-e1	moderate
7	M	14	84	92	19,5	d1-d2	mild
8	F	16	73	97	21,2	d1-d2-g6	moderate
9	M	9	68	61	10,4	d1-d2-g6	moderate
10	M	10	82	90	66,5	g6-w21	mild
11	M	12	98	100	30,2	g6-w21	moderate
12	M	7	100	90	26.5	d1-d2	mild

d1= *Dermatophagoides pteronyssinus*; d2= *Dermatophagoides farinae*; g6= *Phleum pratense*; e1= *Cat epithelium*; w21= *Parietaria judaica*

study (Fig. 2). In particular, a statistically significant decrease during the whole hospitalization period (interval T0-T3) was found for d1 IgE ($p=0.04$), d2 IgE ($p=0.01$) and g6 IgE ($p=0.05$). No significant variations were found for specific IgG4. Specific d1, d2, g6, w21 and e1 IgG4/IgE ratio showed an increase in all the subjects, even if not statistically significant (Fig. 2).

The analysis between T0 and T1 failed to show any significant reduction in specific Ig with the only exception of g6 IgE, which underwent a significant reduction from 35.83 (T0) to 26.00 (T1) kU/L ($p=0.04$).

Considering pooled specific IgE, IgG4 and IgG4/IgE ratio values (sum of specific immunoglobulins for each investigated allergen in the same child),

specific IgE significantly decreased from 113.89 kU/L at T0 to 92.83 kU/L at T3 ($p=0.001$) and from 113.89 kU/L at T0 to 99.52 kU/L at T1 ($p=0.05$) (Fig.3). In contrast, though specific IgG4 increase from T0 to T1, specific IgG4/IgE ratio did not show any statistically significant variation, therefore suggesting that the observed increase in IgG4/IgE may not only be due to the reduction in IgE.

When considering the return of the children to the domestic environment at sea level during the Christmas holidays (T1-T2), no significant variation was demonstrated for specific IgE, IgG4 and IgG4/IgE ratio.

Furthermore, we categorized the data from the sera of the children participating to this study considering the levels of pooled specific immunoglobulins to

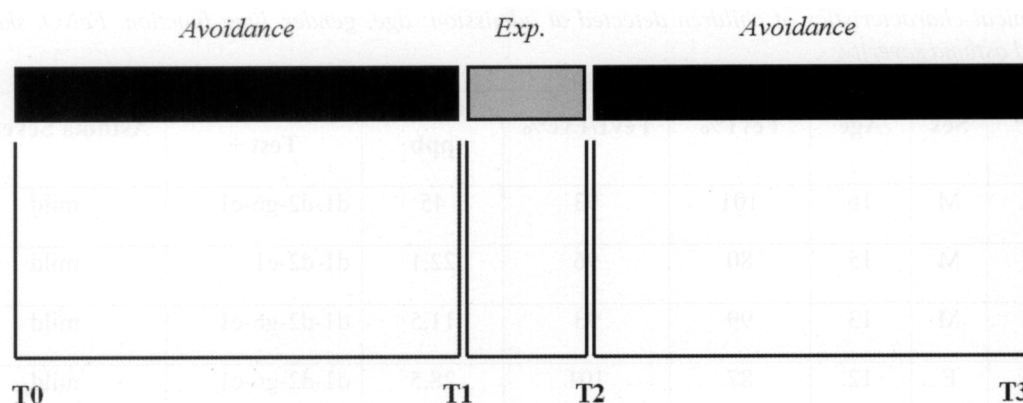


Fig. 1. Experimental design. Avoidance: periods of antigen avoidance. Exp.: period of antigen exposure. T0, T1 (T0 + 96 days), T2 (T0 + 116 days), T3 (T0 + 180 days) correspond to the times of arrival and departure from the Istituto Pio XII (Misurina) when the samples were collected.

allergens to which subjects were skin prick test positive (allergic serums group) and those to which subjects resulted not allergic (not allergic serums group), which served as an internal control. In the allergic serums group, specific IgE decreased from 33.77 kU/L at T0 to 26.33 kU/L at T3 ($p=0.0001$) and from 33.77 kU/L at T0 to 29.52 kU/L at T1 ($p=0.01$). Specific IgG4/IgE ratio increased from 59.60 mg/kU at T0 to 86.84 mg/kU at T3 ($p=0.02$) and from 59.60 mg/kU at T0 to 76.75 mg/kU at T1 ($p=0.02$). In contrast, no IgG4 significant variations were found.

As expected, no significant variations were shown in the not allergic serums group for all the investigated serum markers, therefore supporting the causal role of allergen avoidance for relevant allergens observed in this study design. A positive correlation between specific IgE and specific IgG4 was observed at each considered time (T0: $r=0.57$, $p=0.08$; T1: $r=0.85$, $p=0.001$; T3: $r=0.76$, $p=0.01$) (Fig. 4).

Lung function and FeNO

During the whole period of stay at the residential house, lung function and airway inflammation assessed by FeNO improved. In particular, FEV1 increased from 85.00 Ref% at T0 to 92.75 Ref% ($p=0.006$); FEV1/FVC increased from 89.50 Ref%

at T0 to 92.25 Ref% at T3 ($p=0.01$); FEF 25-75 increased from 66.70 Ref% at T0 to 76.87 Ref% at T3 ($p=0.006$).

FeNO decreased from 26.30 ppb at T0 to 15.11 ppb at T3 (0.01). FeNO showed an increase in consequence of house dust mite re-exposure at home, increasing from 17.43 ppb at T1 to 27.40 ppb at T3 (0.01). A positive correlation between specific IgE and FeNO was found (T0: $p=0.04$ $r=0.63$; T1: $p=0.06$ $r=0.59$; T3: $p=0.02$ $r=0.73$).

DISCUSSION

In this study changes in serum specific IgE and IgG4 were investigated during a 6-month stay in a mite-free environment (16) which was briefly interrupted by a period of natural re-exposure. Antigen avoidance was achieved by a long stay at Istituto Pio XII (Misurina, BL), at 1,756 m above sea level. Routine quantitative assessment of mite and cat major antigens at this site always demonstrated undetectable levels (16).

The study was carried out from mid-September to mid-March, out of season for grass and Parietaria pollens, when the ground is almost constantly covered with snow.

IgG molecules have been described to be involved in immune responses through apparently opposite

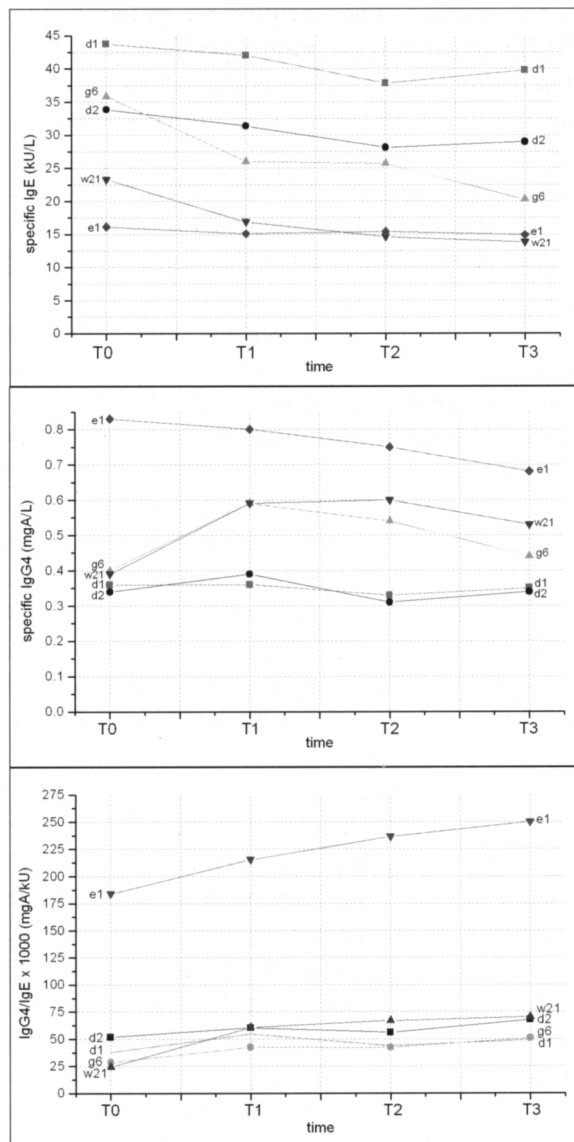


Fig. 2. Time course (T0, T1, T2, T3) of specific IgE, IgG4 and IgG4/IgE ratio mean values. Specific IgE showed a decrease in all the children during the time of the study. No significant variations were found for specific IgG4. Specific IgG4/IgE ratio showed an increase in all the subjects, even if not statistically significant.

mechanisms (26). It has been also demonstrated that minor variations in the IgG-attached sugar moiety can switch an antibody from a pro-inflammatory to an anti-inflammatory state (26). On one hand IgG4 antibodies induce pro-inflammatory responses and recruit innate immune effector cells during infection

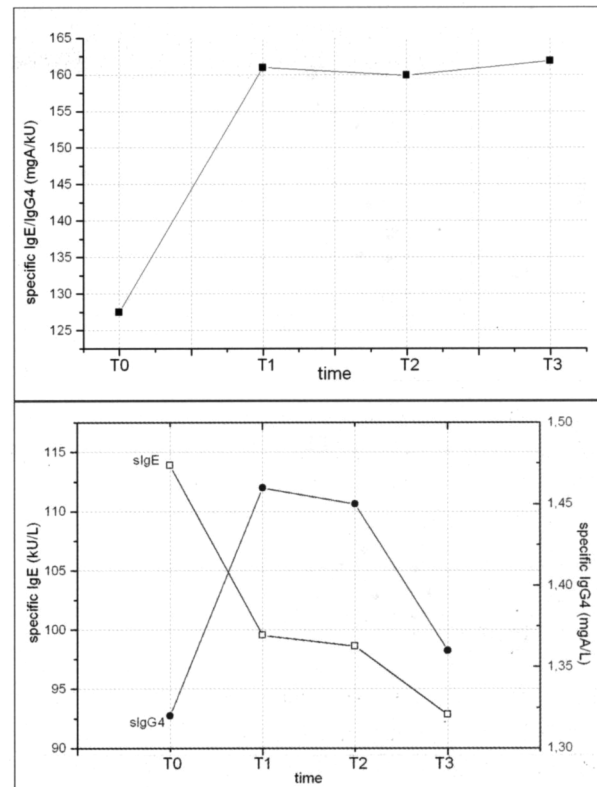


Fig. 3. Mean cumulative levels of specific IgE, IgG4 and IgE/IgG4 time course (T0, T1, T2, T3). Specific IgE significantly decreased from T0 to T3 ($p=0.001$) and from T0 to T1 ($p=0.05$).

with pathogenic microorganisms or autoimmune disease (26). IgG4-related systemic disease has been established as a new systemic disease entity, characterized by high serum IgG4 concentrations and abundant IgG4-bearing plasma cell infiltration in the involved organs (27). The chronic inflammation can involve lacrimal glands, salivary glands, thyroid, lung, pancreas, kidney and prostate. The concept includes Mikulicz'disease, Riedel's thyroiditis, pulmonary fibrosis, pulmonary pseudotumor, autoimmune pancreatitis, a part of tubulo-interstitial nephritis and chronic prostatitis (27). On the other hand, IgG4 seem to play an anti-inflammatory role in immune responses (5-10, 26) which is still largely unexplored in terms of cellular and molecular mechanisms involved in this activity.

It has been suggested that part of the mechanism of specific immunotherapy may involve induction

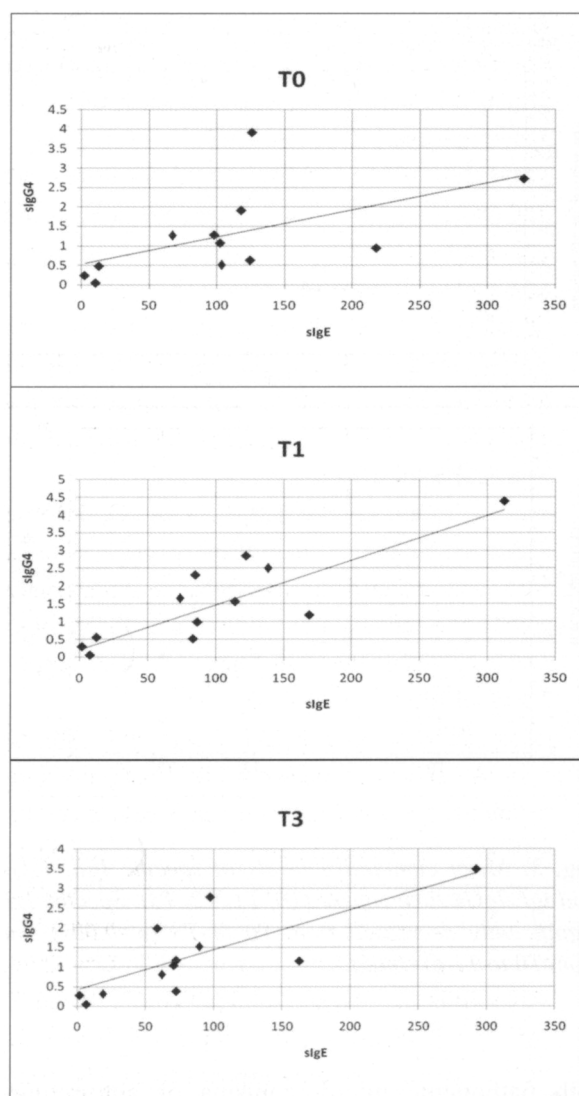


Fig. 4. Relationship between specific IgE and IgG4 (T0, T1, T3). A positive correlation between specific IgE and specific IgG4 was observed at each considered time.

of IgG antibodies that block interaction of allergen and IgE with effector cells (6, 26). Immunotherapy increases specific IgG1 and IgG4 antibody production and promotes down-regulation of IL-4 and IL-13-producing cells in the peripheral blood of treated patients. IgG have been demonstrated to be able to block mast cell activation not only via direct competition for epitope but also via Fc γ RIIB-dependent signals (10, 26).

In fact, it has been demonstrated that IgG-mediated inhibition of mast cell activation could

occur in absence of competition for epitope binding (26). The fact that IgG epitope specificities, which is different from that of the specific IgE, were able to block mast cells activation lead to hypothesize that, in addition to epitope competition between specific IgG and IgE, specific IgG can deliver a negative signal to mast cells. In particular, specific IgG seem to block mast cell activation through Fc γ RIIB (10, 26).

Recent studies have shown that sublingual immunotherapy is associated with a significant increase in allergen-specific IgG4 (28-30). It has also been demonstrated that specific IgG4 serum levels are correlated with the improvement in the airway response to allergen challenge (28).

To the best of our knowledge, no study has evaluated the effect of antigen avoidance on IgG4 serum levels in asthmatic children. In this study all the patients showed a generalized decrease in serum specific IgE during their stay at the residential house in Misurina, with a different kinetic for each allergen, in keeping with previous reports (13-14). No significant variations were found for specific IgG4, while in all subjects an increase of allergens specific IgG4/IgE ratio was demonstrated. Specific IgG4 did not show a common trend either between the patients or between the considered allergens. Nevertheless, considering the subjects individually, we found a consensual trend between house dust mites IgG4 and pollens IgG4 which was possibly related to the events of this experimental design. Though these data might, therefore, suggest that individual factors could influence the IgG4 trend, the low number of subjects considered in this study does not allow any further statistical approach to finalize a more detailed insight.

During the brief time spent in their domestic environment at sea level for the Christmas holidays, the children were presumably re-exposed to different levels of mite antigens, whereas, during this period (end of December - begin of January) the children were not exposed to considerable pollen concentrations. Unfortunately, the design of this pilot study did not consider the measurement of the effective re-exposure to cat allergen. When considering the return of the children to the domestic environment at sea level during the Christmas holidays (T1- T2), no significant variation was demonstrated for specific IgE, IgG4 and

IgG4/IgE ratio. A possible explanation for the lack of a significant change at these time points could be the very short period of allergen re-exposure (14).

As expected on the basis of previous studies (15-16) during the whole period of stay at the residential house, lung function and airway inflammation assessed by FeNO improved while a worsening after dust mite re-exposure was demonstrated only for FeNO.

Furthermore, a significant correlation was found between specific IgE and specific IgG4, thus suggesting that the two classes of immunoglobulins may be somehow dependent on each other. The demonstration that the specific IgG4/IgE ratio is increased, even if not significantly, during the period of antigen avoidance might suggest the prevalence of anti-inflammatory response following allergen removal, paralleling the improvement of clinical symptoms and the reduction in FeNO.

In conclusion, the results of this study, with a positive correlation between specific IgE and specific IgG4 observed at all the times of study, suggest a relationship between IgE and IgG4 in response to environmental factors, though the actual role of IgG4 in the mechanisms underlying natural antigen avoidance in asthmatic children cannot be explained by the results of the present study. Further studies are therefore warranted to have a deeper insight of the role of IgG4 antibodies in the mechanisms of antigen avoidance and exposure in allergic asthmatic children.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Staffan Ahlstedt and Phadia AB, Uppsala for providing the dosages of IgE and IgG4 in the sera of the patients.

REFERENCES

1. Jenmalm MC, Bjorksten B. Development of immunoglobulin G subclass antibodies to ovalbumin, birch and cat during the first eight years of life in atopic and non-atopic children. *Pediatr Allergy Immunol* 1999; 10:112-21.
2. Boettcher M, Jenmalm M, Bjorksten B. Immune response to birch in young children during their first 7 years of life. *Clin Exp Allergy* 2002; 32:1690-98.
3. Jabara HH, Loh R, Ramesh N, Vercelli D, Geha RS. Sequential switching from mu to epsilon via gamma 4 in human B cells stimulated with IL-4 and hydrocortisone. *J Immunol* 1993; 151:4528-33.
4. Vercelli D, De Monte L, Monticelli S, Di Bartolo C, Agresti A. To E or not to E? Can an IL-4-induced B cell choose between IgE and IgG4? *Int Arch Allergy Immunol* 1998; 116:1-4.
5. Witteman AM, Stapel SO, Sjamsoedin DH, Jansen HM, Aalberse RC, der Zee JS. Fel d 1-specific IgG antibodies induced by natural exposure have blocking activity in skin tests. *Int Arch Allergy Immunol* 1996; 109:369-75.
6. Van Neerven RJ, Wikborg T, Lund G, Jacobsen B, Brinch-Nielsen A, Arnved J, Ipsen H. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4+ T cells by inhibiting serum-IgE-facilitated allergen presentation. *J Immunol* 1999; 163:2944-52.
7. Rowntree S, Platts-Mills TAE, Cogswell JJ, Mitchell EB. A subclass IgG4-specific antigen-binding radioimmunoassay (RIA): comparison between IgG and IgG4 antibodies to food and inhaled antigens in adult atopic dermatitis after desensitization treatment and during development of antibody responses in children. *J Allergy Clin Immunol* 1987; 80:622-30.
8. Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitization, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 2001; 357:752-56.
9. Matsui EC, Diette GB, Krop EJ, Aalberse RC, Smith AL, Curtin-Brosnan J, Eggleston PA. Mouse allergen-specific immunoglobulin G and immunoglobulin G4 and allergic symptoms in immunoglobulin E-sensitized laboratory animal workers. *Clin Exp Allergy* 2005; 35:1347-53.
10. Uermösi C, Beerli RR, Bauer M, Manolova V, Dietmeier K, Buser RB, Kündig TM, Saudan P, Bachmann MF. Mechanisms of allergen-specific desensitization. *J Allergy Clin Immunol* 2010; 126: 375-83.
11. Charpin D, Kleisbauer JP, Lanteaume A, Razzouk H, Vervloet D, Toumi M, Faraj F, Charpin J. Asthma and allergy to house-dust mites in populations living in high altitudes. *Chest* 1988; 93:758-61.

12. Charpin D, Birnbaum J, Haddi E, Genard G, Lanteaume A, Toumi M, Faraj F, Van der Brempt X, Vervloet D. Altitude and allergy to house-dust mites. A paradigm of the influence of environmental exposure on allergic sensitization. *Am Rev Respir Dis* 1991; 143: 983-86.
13. Bodini A, Peroni D, Vicentini L, et al. Exhaled breath condensate eicosanoids and sputum eosinophils in asthmatic children: a pilot study. *Pediatr Allergy Immunol* 2004; 15:26-31.
14. Sensi LG, Piacentini GL, Nobile E, Ghebregzabher M, Brunori R, Zanolli L, Boner AL, Marcucci F. Changes in nasal specific IgE to mites after periods of allergen exposure-avoidance: a comparison with serum levels. *Clin Exp Allergy* 1993; 24:377-82.
15. Piacentini LG, Bodini A, Costella S, Vicentini L, Peroni P, Zanolli L, Boner AL. Allergen avoidance is associated with a fall in exhaled nitric oxide in asthmatic children. *J Allergy Clin Immunol* 1999; 104:1323-24.
16. Boner AL, Comis A, Schiassi M, Venge P, Piacentini GL. Bronchial reactivity in asthmatic children at high and low altitude. Effect of budesonide. *Am J Respir Crit Care Med* 1995; 151:1194-200.
17. Grootendorst DC, Dahlén SE, Van Den Bos JW, et al. Benefits of high altitude allergen avoidance in atopic adolescents with moderate to severe asthma, over and above treatment with high dose inhaled steroids. *Clin Exp Allergy* 2001; 31:400-8.
18. [No authors listed]: Skin test in type I allergy testing Position paper. Sub-Committee on skin Tests of the European Academy of Allergology and Clinical Immunology. *Allergy* 1989; 44(S):1-59.
19. Piacentini GL, Peroni DG, Bodini A, Pigozzi R, Costella S, Loiacono A, Boner AL. Azithromycin reduces bronchial hyperresponsiveness and neutrophilic airway inflammation in asthmatic children. A preliminary report *Allergy and Asthma Proceedings* 2007; 28:194-98.
20. Piacentini GL, Bodini A, Peroni DG, Miraglia Del Giudice M, Costella S, Boner AL. Reduction in exhaled nitric oxide immediately after methacholine challenge in asthmatic children. *Thorax* 2002; 57: 771-73.
21. Evans G, Rilven M. Bulk Reagent Random-Access analyzers: UniCAP 100. In: *The Immunoassay Handbook*. Frome, UK: Tradespools Ltd 2000.
22. Cressy I, Magnusson B, Soderstrom L, Kober A. IgG4 antibody concentrations to cat and mite allergens measured with the new ImmunoCAP specific IgG4 method. In: *Proc XXV Congress of the European Academy of Allergy and Clinical Immunology*. Vienna Austria 2006: 296.
23. Wood RA, Segall N, Ahlstedt S, Williams PB. Accuracy of IgE antibody laboratory results. *Ann Allergy Asthma Immunol* 2007; 99:34-41.
24. Cain TW, Ford J, Dolen WK. A low-level method for measurement of specific immunoglobulin E antibody serum. *Allergy Clin Immunol Int J World Allergy Org* 2006; 18/6:1-4.
25. Sasai K, Furukawa S, Muto T, Baba M, Yabuta K, Fukuwatari Y. Early detection of specific IgE antibody against house dust mite in children at risk of allergic disease. *J Pediatr* 1996; 128:834.
26. Aschermann S, Lux A, Baerenwaldt A, Biburger M and Nimmerjahn F. The other side of immunoglobulin G: suppressor of inflammation. *Clin Exp Immunol* 2009; 160:161-67.
27. Yamamoto M, Takahasashi H, Shinomura Y. IgG4-related systemic disease/systemic IgG4-related disease. *Rinsho Byori* 2010; 58:454-65.
28. Peroni DG, Piacentini GL, Bodini A, Boner AL. Snail anaphylaxis during house dust mite immunotherapy. *Pediatr Allergy Immunol* 2000; 11:260-61.
29. Piconi S, Trabattoni D, Rainone V, Borgonovo L, Passerini S, Rizzardini G, Frati F, Iemoli E, Clerici M. Immunological effects of sublingual immunotherapy: clinical efficacy is associated with modulation of programmed cell death ligand 1, IL-10 and IgG4. *J Immunol* 2010; 185:7723-30.
30. Bush RK, Swenson C, Fahlberg B, Evans D, Esch R, Busse WW. House dust mite sublingual immunotherapy: Results of a US trial. *J Allergy Clin Immunol* 2011; 127:974-81.