

IMMUNOGLOBULIN PRODUCTION PATTERN IS ALLERGEN-SPECIFIC IN POLYSENSITIZED PATIENTS

G. CIPRANDI, M. DE AMICI¹, M.A. TOSCA², S. NEGRINI, F. PUPPO
and G.L. MARSEGLIA¹

Department of Internal Medicine, University of Genoa, Genoa; ¹Pediatric Clinic, Foundation IRCCS San Matteo, University of Pavia, Pavia; ²Pneumology and Allergology, IRCCS G. Gaslini, Genoa, Italy

Received February 26, 2009 – Accepted May 13, 2009

Allergic rhinitis (AR) is characterized by Th2 polarized immune response, such as increased IL-4 and reduced IFN- γ production, and by a functional defect of T regulatory cells. This impaired immune response profile influences the pattern of immunoglobulin production in allergic patients. Therefore, the aim of this study is firstly to investigate the allergen-specific IgE, IgG, IgG4, and IgA serum level pattern in polysensitized AR patients with the same skin prick test positivity to some pollen allergens. Secondly, this study aims at relating immunoglobulin (Ig) values with some clinical and immunological parameters. Eighty polysensitized patients with AR were enrolled. Serum allergen-specific IgE, IgG, IgG4, and IgA for mites, Parietaria, grasses, and birch, TGF- β and sHLA-G were determined by the ELISA method. Allergic symptoms and drugs use were also assessed. Allergen-specific IgE, IgG, IgG4, and IgA serum levels were significantly different for each tested allergen ($p=0.0001$). There was a significant correlation between IgE levels and allergy severity, whereas IgA had an antagonistic behaviour, considering mite-specific immunoglobulins. In conclusion, the present study provides the first evidence that immunoglobulin production pattern depends on the specificity of the allergenic response.

Allergic rhinitis (AR) represents the most common immune-mediated disorder, and its prevalence is increasing worldwide (1). AR is sustained by an IgE-dependent inflammation, mainly consisting of mucosal infiltration by activated mast cells and eosinophils. This inflammatory reaction displays a predominant interleukin (IL)-4 production by Th2 cells over interferon (IFN)- γ expression by Th1 cells: the so-called Th2 polarization (2). Th2-dependent cytokines, including IL-4 and IL-13, promote, amplify, and maintain the ongoing production of allergen-specific IgE, such as the typical phenomenon occurring in

allergic patients. Furthermore, a number of recent studies have indicated that regulatory T cells (Tregs) play an important role in controlling the Th2-biased responses in allergic patients (3-6). Indeed, a role for T regulatory cells has been evidenced in patients treated with allergen-specific immunotherapy for grasses and house dust mites (4). On the basis of these findings, it has been pointed out that successful immunotherapy is capable of modifying the T cell response to allergen through the induction of regulatory mechanisms that are defective in untreated allergic patients (7). Indeed, the normal healthy response to allergens is

Key words: allergic rhinitis, allergens, serum, IgG, IgG4, IgA, IgE, TGF- β , sHLA-G, allergy severity

Mailing address: Giorgio Ciprandi, M.D.
Semeiotica e Metodologia Medica I, DIMI,
Viale Benedetto XV 6,
16132 Genoa, Italy
Tel: ++39 010 35338120
Fax: ++39 010 5556696
e-mail: gio.cip@libero.it

characterized by suppression of allergen-specific Th2 responses by T regulatory cells. This issue appears crucial, as a functional defect of T regulatory cells has been demonstrated by an experimental study in allergic subjects (8). In this regard, the production of inhibitory cytokines, such as IL-10 and TGF- β , is thought to be relevant for the function of T regulatory cells. IL-10 exerts numerous anti-inflammatory activities, including down-regulation of both Th1 and Th2 cytokine production and anergy of effector T cells, and also increases allergen-specific IgG4 production, inhibiting IgE synthesis. Also TGF- β has multiple regulatory effects on T cell proliferation, antigen presentation, and expression of co-stimulatory proteins (9). In addition, TGF- β drives the production of IgA (10). Successful subcutaneous immunotherapy is followed by increased production of both IL-10 and TGF- β . Furthermore, an increased IgG4 and IgA production has been demonstrated to be associated with IL-10 and TGF- β production (10).

The different response to allergens between normal subjects and allergic patients may substantially consist in the class pattern of produced allergen-specific immunoglobulins: IgG and IgA in normal subjects, IgE in allergic patients. Indeed, immunotherapy may hence restore a normal T regulatory cell function that consists in inducing an allergen-specific clinical tolerance as provided by restored IgG and IgA production.

Moreover, sensitization to more than one allergen (the so called polysensitization) is a very frequent phenomenon and the skin prick test is not sufficient to define the causal relevance of each sensitizing allergen. In this regard, there is no study that investigated the pattern of immunoglobulins considering the specificity of causal allergens in polysensitized patients with allergic rhinitis.

The aims of this study are: i) to investigate the pattern of allergen-specific IgE, IgG (total and IgG4), and IgA production with relation to allergen specificity, ii) and to relate it with some clinical and immunological parameters in polysensitized AR patients.

MATERIALS AND METHODS

Study design

The study evaluated 80 polysensitized AR patients, 47 females and 33 males; the median age of allergic patients was 35 years (range 18-71). All were consecutively

evaluated when they were symptomatic at the end of the pollen season. The skin prick test and blood sampling for assessing serum immunoglobulins and mediator levels were performed in all subjects. All patients had to have a diagnosis of pollen-induced persistent allergic rhinitis and skin prick test positive (+++++) for *Parietaria*, grasses, and birch to be included in the study. Exclusion criteria included: concomitant asthma and/or rhinosinusitis, prior or actual allergen-specific immunotherapy, duration of allergic rhinitis longer than 5 years, other acute or chronic disorders.

The study was approved by the local Ethics Committee and written informed consent was given by all participants. Allergic rhinitis was diagnosed according to validated criteria (11). Briefly, the diagnosis of allergic rhinitis was made on the basis of a positive history of nasal symptoms (i.e. nasal itching, sneezing, watery rhinorrhea, and nasal obstruction) during the pollen season for at least 2 years and positive (+++++) skin prick test for pollen allergens.

Skin prick test

This assay was performed according to the guidelines of the European Academy of Allergy and Clinical Immunology (12). The panel of allergens employed consisted of: house dust mites (*Dermatophagoides farinae* and *pteronyssinus*), cat, dog, grass mix, *Compositae* mix, *Parietaria officinalis*, birch, hazel, olive tree, *Alternaria tenuis*, *Cladosporium*, *Aspergilli* mix (Stallergenes, Milan, Italy).

Clinical assessment

Clinical severity was also evaluated, considering nasal symptom perception and drug consumption at the end of the pollen season, as previously validated (13). The patient questionnaire included a validated visual analogue scale (VAS) for quantifying the perception of nasal symptom intensity (16). Patients had to have a VAS score for nasal symptoms > 5.

During the pollen season, use of levocetirizine drops (ten or twenty, corresponding to a 2.5 or 5 mg concentration) on demand as rescue medication was permitted. However, medication was withdrawn at least one week before taking blood samples. The medication scoring (MS) was as follows: not at all (0), occasionally/seldom (1), occasionally/often (2), almost daily (3), continuously with the lowest dose (4), continuously with the maximal dose (5).

Specific IgE

Allergen-specific IgE ImmunoCAP (Phadia) was measured using the Fluorezyme-immunoassay (FEIA). The allergen of interest, covalently coupled to ImmunoCAP, reacts with the specific IgE in the patient

serum specimen. After washing away non-specific IgE, enzyme labeled antibodies against IgE were added to form a complex. After incubation, unbound enzyme-anti-IgE was washed away and the bound complex was then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate was measured. The higher the response value, the more specific IgE was present in the specimen. To evaluate the test results, the responses for the patient samples were transformed to concentrations with the use of a calibration curve (0-100 kU/l).

ImmunoCAP Specific IgE Calibrators were used for determination of specific IgE antibodies and values are expressed in kU/l. Values ≥ 0.35 kU/l represent a progressive increase in the relative concentration of allergen-specific antibodies. Results < 0.35 kU/l represent absence or undetectable levels of allergen-specific antibodies.

Specific IgG and IgG4

Determination of allergen-specific IgG4 and total IgG antibodies was performed on all sera against the causal allergens: *Betula alba*, grasses, and *Parietaria judaica* in a single laboratory session (Phadia AB, Uppsala, Sweden) using an IgG or IgG4 ImmunoCAP FEIA assay described previously (14). Briefly, the method consisted of the following steps: the antigen of interest, covalently coupled to ImmunoCAP, reacted with the specific IgG (IgG4) antibodies in the diluted patient serum specimen. After washing away non-specific IgG (IgG4), enzyme labeled antibodies against IgG (IgG4) were added to form a complex. After incubation, unbound enzyme-anti-IgG (IgG4) was washed away and the bound complex was then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate was measured. The higher the response value, the more specific IgG (IgG4) antibodies were present in the specimen. To evaluate the test results, the response for the patient samples were transformed to concentrations with the use of a calibration curve (Specific IgG: 1:100 diluted sample 2-200 mg_A/l; Specific IgG4: 0-300 µg_A/l; where A represents antigen-specific antibodies). Before assaying, all serum have to be diluted 1:100 with Specific IgG (IgG4) Sample Diluent. The dilution step can be performed manually or automatically using ImmunoCAP 100. ImmunoCAP Specific IgG (IgG4) Calibrator Strip was used for determination of total IgG (IgG4) and values were expressed in mg/L (mg/l). The detection limit for IgG was < 0.02 mg_A/L. The limit of quantization for antigen specific IgG4 antibodies was 0.07 µg/L.

Specific IgA

Specific IgA ImmunoCAP (Phadia) was experimentally

used for research purposes only (measured for those pollens and mites, using the Fluorezyme immunoassay (FEIA). The calibrators are traceable to the International Reference Preparation (IRP) 67/86 for Human Serum Immunoglobulins A, G and M from World Health Organization (WHO). The measuring range for a diluted sample is 1.0-100 mg_A/l (where A represents antigen-specific antibodies). The detection limit is < 0.01 mg_A/l.

Serum TGF-β immunoassay

The human Transforming Growth Factor beta 1 (TGF-β1) Immunoassay (Quantikine TGF-β Immunoassay, R&D Systems Inc. Italy, Milan) employs the quantitative sandwich enzyme immunoassay technique and was performed according to the manufacturer's instructions and expressed as ng/mL.

Soluble HLA-G molecule immunoassay

sHLA-G molecules were determined by a commercially available immunoenzymatic assay (EXBIO, Praha, Czech Republic) which was recently validated in a workshop (15) according to the manufacturer's instructions, and results were expressed as ng/mL.

Statistical analysis

Descriptive statistics were first performed and quantitative parameters were reported as median (md). The non-parametric Wilcoxon test was used to compare samples. The non-parametric Kruskal-Wallis rank test was performed to evaluate the analysis of variance between groups of allergens. The correlation between quantitative differences was evaluated by the non-parametric Spearman's correlation coefficient (r_s). For the purpose of this analysis, correlations coefficients ≥ 0.8 were considered very strong, from 0.6 to 0.79 were considered strong, from 0.4 to 0.59 were considered moderate, from 0.2 to 0.39 were considered weak and less than 0.2 were considered very weak (17).

All tests were two sided and a p-value less than 0.05 was considered statistically significant. The package "S-Plus" (MathSoft Corp) was used for all the analyses.

RESULTS

All patients were sensitised and allergic to *Betula alba*, *Parietaria judaica*, and *Graminaceae* (ten of them were also sensitised and allergic to *Dermatophagoides farinae* and *pteronysinus*) with the same skin prick test result for all allergens, such as ++++.

Fig. 1 shows that the sum of the measured allergen-specific Ig classes is significantly dependent

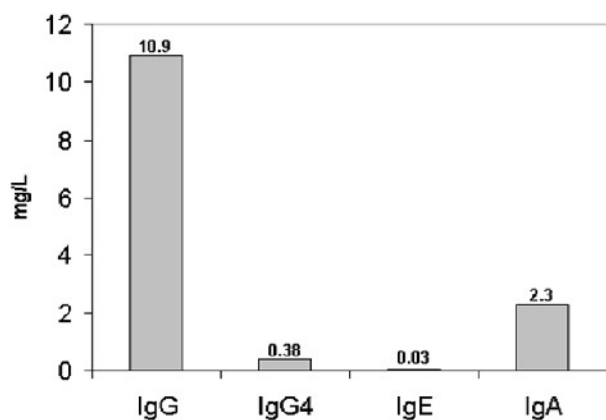


Fig. 1. Ig class (IgE, IgA, IgG, IgG4) distribution in patients without allergen distinction. Values are represented as Median.

on the singular class. Moreover, IgE shows the lowest concentration.

As shown in Fig. 2, specific IgE levels significantly changed (Kruskal-Wallis test; $p=0.0001$) among the groups of allergens; in particular, there was a significant difference of IgE levels between mites (md: 6.89 kU/L) and Parietaria (md: 50.05 kU/L) (Wilcoxon test; $p=0.0034$), between birch (md: 6.96 kU/L) and Parietaria (Wilcoxon test; $p=0.0001$) and between grasses (md: 1.51 kU/L) and Parietaria (Wilcoxon test; $p=0.0001$).

Specific IgA levels significantly changed (Kruskal-Wallis test; $p=0.0433$) among the groups of allergens; in particular, there was a significant difference of IgA levels between mites (md: 3.49 mg/L) and grasses (md: 1.68 mg/L) (Wilcoxon test; $p=0.0104$), and between mites and Parietaria (md: 2.2 mg/L) (Wilcoxon test; $p=0.033$), but there was no significant difference between mites and birch (md: 2.94 mg/L) (Wilcoxon test; $p=0.0826$) (Fig. 2).

Specific IgG levels significantly changed (Kruskal-Wallis test; $p=0.0001$) among the groups of allergens; in particular, there was a significant difference of IgG levels between mites (md: 24.35 mg/L) and grasses (md: 8.26 mg/L) (Wilcoxon test; $p=0.0001$), between mites and birch (md: 3.75 mg/L) (Wilcoxon test; $p=0.0001$), between mites

and Parietaria (md: 16.9 mg/L) (Wilcoxon test; $p=0.0129$), between grasses and birch (Wilcoxon test; $p=0.0001$), between grasses and Parietaria (Wilcoxon test; $p=0.0001$) and between Parietaria and birch (Wilcoxon test; $p=0.0001$) (Fig. 2).

Specific IgG4 levels significantly changed (Kruskal-Wallis test; $p=0.0433$) among the groups of allergens; in particular, there was a significant difference of IgG4 levels between mites (md: 0.45 mg/L) and grasses (md: 0.145 mg/L) (Wilcoxon test; $p=0.0024$), between mites and birch (md: 0.16 mg/L) (Wilcoxon test; $p=0.057$), between grasses and Parietaria (md: 0.65 mg/L) (Wilcoxon test; $p=0.0001$) and between Parietaria and birch (Wilcoxon test; $p=0.0001$) (Fig. 2).

As shown in Fig. 3, for the allergen mites, there was a strong correlation between IgE levels and Age ($r=-0.66$) and between IgE levels and VAS ($r=0.689$). There was a moderate correlation between IgE levels and Eosinophil Counts ($r=0.5$), sHLA-G ($r=0.45$) and Medication Scoring ($r=0.447$).

As shown in Fig. 4, for the allergen mites, there was a strong correlation between IgA levels and TGF-beta serum levels ($r=0.733$). There was a moderate correlation between IgA levels and VAS ($r=-0.403$) and between IgA levels and Medication Scoring ($r=-0.48$).

DISCUSSION

Th2-derived cytokines, such as IL-4 and IL-13, are the primary pathogenic factors in inducing, maintaining, and amplifying allergic inflammation. As a matter of fact, IL-4 and IL-13 orchestrate allergic inflammation promoting IgE synthesis, up-regulating adhesion molecules selective for eosinophil recruitment, and causing increased mucus production and airway hyperreactivity (18). The production of allergen-specific IgE may be considered as the *stigmata* of allergic reaction, namely it may be considered as the *conditio sine qua non* for determining allergy. Recently, it has been evidenced that each allergen is able to induce an immune response both in allergic patients and in normal subjects: this phenomenon is fairly obvious as the allergen is an antigen (8). However, the type of response is different between allergic patients and normal subjects as it resides in the different

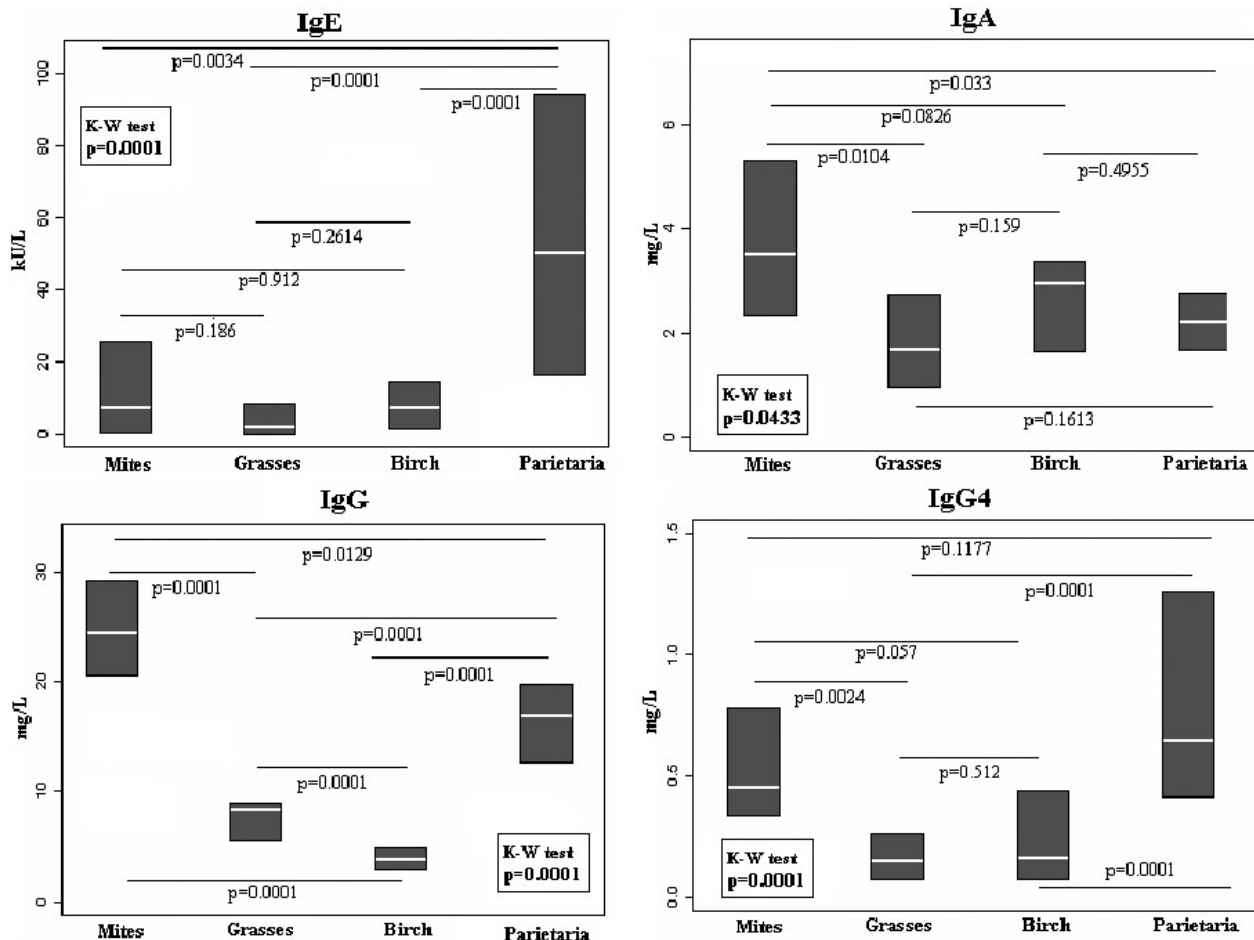


Fig. 2. Serum allergen specific IgE, IgA, total IgG, and IgG4 distribution in patients evaluated for the single allergens. Values are represented as medians (white line), quartiles (25th and 75th percentiles, black box), and p-values between the groups.

Immunoglobulin pattern: IgG and IgA in normal subjects and IgE in allergic ones (8). The reason for this difference is based on a functional defect of T regulatory cells that normally direct toward a physiological IgG-IgA response. Thus, allergic patients have a functional defect of Tregs and polarized Th2 response with ongoing IgE synthesis (7).

Allergic rhinitis may be characterized by a negative evolution, mainly consisting in: worsening of allergy degree, involvement of bronchi, and polysensitization (19). Indeed, most allergic patients are polysensitized (20). This fact may have relevant clinical implications, such as the allergen choice for immunotherapy (21). However, there is no study,

which has investigated the pattern of immunoglobulin production in polysensitized patients considering the different allergens.

This study firstly shows that IgE serum levels are the lowest in comparison with the other classes: the reasonable explanation is that IgE are cytophilic, as IgE are mainly placed in tissues. Secondly, this study provides evidence that the pattern of the different classes of allergen-specific immunoglobulins is strictly and significantly dependent on the type of allergen, as the immune response is quantitatively specific for each allergen and is deeply dependent on the singular class. Indeed, each Ig class shows a different production degree for each single tested allergen.

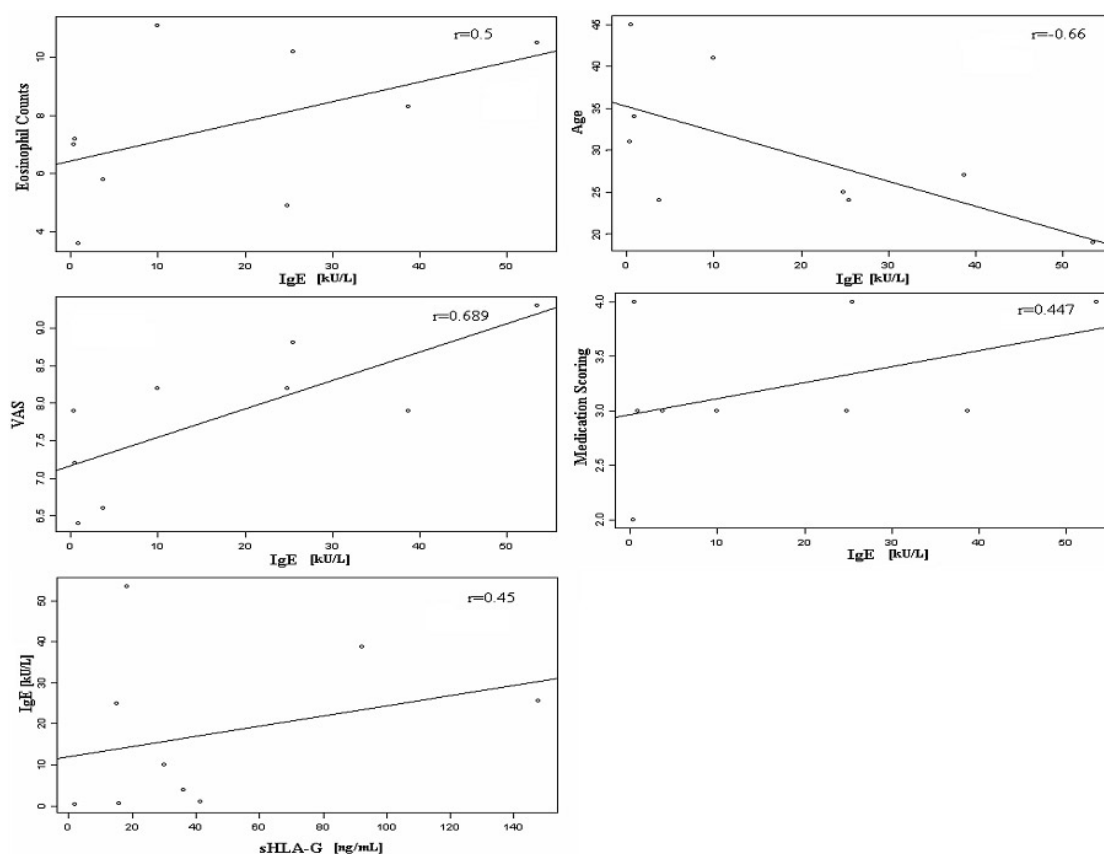


Fig. 3. Correlation, expressed as Spearman's correlation coefficient (r), between IgE serum levels and some clinical and immunological parameters.

In particular, *Parietaria* allergen induces the most relevant quantity of IgE. On the other hand, mites induce the highest quantity of specific IgA as well as of specific total IgG, followed by *Parietaria*, grasses, and birch. *Parietaria* and mites are the most important inducers of IgG4 production. These events might depend at least on two factors. Firstly, the allergenic property, such as the capacity of inducing and stimulating IgE synthesis, that is specific for each allergen. Secondly, the duration of allergen exposure: maximal for mites and *Parietaria*. In fact, a prolonged exposure to the causal allergen may amplify a persistent inflammatory response that may both maintain a Th2 polarization and induce an attempt of tolerance by T regulatory cells. In this regard a recent study demonstrated that TGF- β production depends on allergen exposure (manuscript submitted).

Another relevant finding is the inverse relationship between IgE levels and age. This phenomenon supports the common perception that allergic disorders tend to improve with age. IgE levels are significantly related with allergic inflammation, as shown by the significant relationship with eosinophils. In addition, IgE levels are significantly related with allergic symptom severity and drug use. Interestingly, IgE levels are significantly related with serum soluble HLA-G levels: sHLA-G has been recently demonstrated to be a biomarker of allergy severity (22-23). Allergen-specific IgE levels may be a good marker for assessing allergy.

On the other hand, IgA levels are significantly related to the TGF- β levels. This finding confirms that IgA production depends on the function of T regulatory cells. IgA levels are inversely related with allergic symptom severity and drug use. These

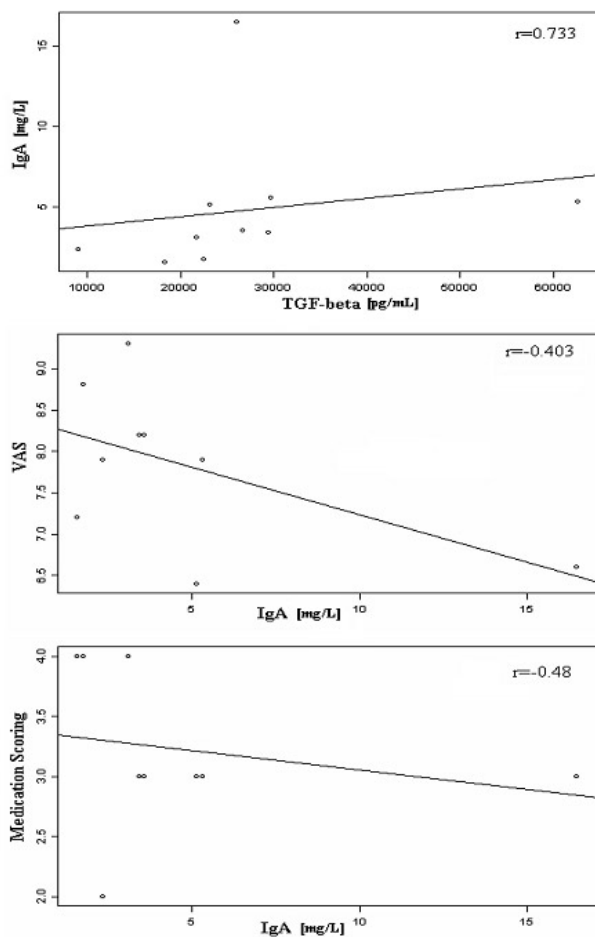


Fig. 4. Correlation, expressed as Spearman's correlation coefficient (r), between IgA serum levels and some clinical and immunological parameters.

findings could support the concept that IgA may play a “protective” role. It might be considered that there is a functional dichotomy between IgE and IgA production: the first depending on Th2 polarization, the second depending on T regulatory cell tolerogenic functions. In addition, it is of note that all patients were polysensitized and allergic to all pollens with the same positivity degree at skin prick test. This fact underlines that a skin prick test is not sufficient to detect the real degree of allergy as it is fundamentally a “qualitative” test and is not able to discriminate quantitative differences between allergens.

The most relevant and original finding of this study is represented by the fact that it provides

the first evidence showing that allergen-specific immunoglobulin pattern production is allergen-dependent in polysensitized patients. There are allergens that better induce an IgE response, as well as Tregs functions that may depend on the type of allergen and the exposure duration. On the other hand, the specificity of the immune response to allergens has been previously indicated as some studies were addressed to this issue. A first study reported that both inflammatory degree and nasal airflow are related to the specific causal allergen (24). This study therefore highlights that each single allergen is able to induce a distinct immune and clinical response. Secondly, it has been reported that pollen-induced persistent allergic rhinitis is different from mite-induced AR in nasal airflow, such as pollen-induced AR is characterized by more impaired nasal airflow than mites-induced AR (25). Finally, it has been demonstrated that the *in vitro* production of IFN- γ depends on the specific allergen used as stimulus (26).

Thus, all these studies clearly provide evidence that each allergen induces a specific response that translates into different clinical, functional, and immunological effects. The clinical relevance of this study is based on the fact that all patients were sensitized to 3 pollens with the same degree of skin prick test response. In other words, the skin prick test is insufficient for careful evaluation of IgE production. In conclusion, this study evidenced that the immunoglobulin pattern is allergen-specific in allergic patients presenting with polysensitisation. In addition, IgE are related to clinical severity and IgA seem to antagonize it. These results may therefore be considered clinically relevant proof of the specificity of allergic reaction as the Ig response patterns depend on the specific allergen. These preliminary findings need to be confirmed by further studies conducted on non-allergic subjects and in patients treated with immunotherapy.

ACKNOWLEDGEMENTS

The authors wish to thank Cristina Torre and Giuseppe Mantegna (Clinica Pediatrica, Fondazione IRCCS Policlinico S. Matteo) for outstanding technical support, and to Vania Giunta (Dipartimento di Informatica e Sistemistica, Università di Pavia) for data analysis.

REFERENCES

1. Plaut M, Valentine MD. Allergic rhinitis. *N Engl J Med* 2005; 353:1934-44.
2. Finkelman FD, Vercelli D. Advances in asthma, allergy mechanisms, and genetics in 2006. *J Allergy Clin Immunol* 2007;120:544-50.
3. Umetsu DT, DeKruyff RH. The regulation of allergy and asthma. *Immunol Rev* 2006;212:238-55.
4. Francis JN, Till SJ, Durham SR. Induction of IL10+CD4+CD25+ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol* 2003;111:1255-61.
5. Savolainen J, Laaksonen K, Rantio-Lehtimäki A, Terho EO. Increased expression of allergen-induced *in vitro* interleukin-10 and interleukin-18 mRNA in peripheral blood mononuclear cells of allergic rhinitis patients after specific immunotherapy. *Clin Exp Allergy* 2004;34:413-9.
6. Blaser K, Akdis CA. Interleukin-10, T regulatory cells and specific allergy treatment. *Clin Exp Allergy* 2004;34:328-31.
7. Ciprandi G, Fenoglio D, Cirillo I, et al. Induction of interleukin 10 by sublingual immunotherapy for house dust mites: a preliminary report. *Ann Allergy Asthma Immunol* 2005;95:38-44.
8. Ling EM, Smith T, Nguyen XD, et al. Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet* 2004;363:608-15.
9. Jutel M, Akdis M, Budak F, et al. IL10 and TGFβ cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003;33:1205-14.
10. James LK, Durham SR. Update on mechanisms of allergen injection immunotherapy. *Clin Exp Allergy* 2008;38:1074-88.
11. Bousquet J, Khaltayev N, Cruz AA, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 Update (in collaboration with the World Health Organization, GA2LEN and AllerGen). *Allergy* 2008;63(S):8-160.
12. Dreborg S, Backman A, Basomba A. Skin tests used in type I allergy testing. Position paper. *Allergy* 1989;44(S):1-59.
13. Bousquet PJ, Combescure C, Neukirch F, Klossek JM, Mechin H, Daures JP, Bousquet J. Visual analog scales can assess the severity of rhinitis graded according to ARIA guidelines. *Allergy* 2007;62:367-72.
14. Meier P, Muller U. Evaluation of IgG RAST FEIA for the assay of venom-specific IgG antibodies during venom immunotherapy. *Int Arch Allergy Immunol* 1998;117:46-51.
15. Rebmann V, Lemaoult J, Rouas-Freiss N, Carosella ED, Grosse-Wilde H. Report of the wet workshop for quantification of soluble HLA-G in Essen, 2004. *Hum Immunol* 2005;66:853-63.
16. Ciprandi G, Contini P, Fenoglio D, Sormani MP, Negrini S, Puppo F, Indiveri F. Relationship between soluble HLA-G and HLA-A,-B,-C serum levels and IFN-gamma production after sublingual immunotherapy in patients with allergic rhinitis. *Human Immunology* 2008;69:510-2.
17. Swinscow TDV. Statistics at square one. Ninth Edition, Revised by Campbell MJ, University of Southampton, BMJ publishing Group, 1997.
18. Tang C, Inman MD, van Rooijen N, et al. Th type-1-stimulating activity of lung macrophages inhibits Th2-mediated allergic airway inflammation by an IFN-gamma-dependent mechanism. *J Immunol* 2001;166:1471-81.
19. Ciprandi G, Cirillo I, Pistorio A. Impact of allergic rhinitis on asthma: effects on spirometric parameters. *Allergy* 2008;63(3):255-60.
20. Ciprandi G, Cirillo I, Vizzaccaro A, et al. Seasonal and perennial allergic rhinitis: is this classification adherent to real life? *Allergy* 2005;60:882-7.
21. Ciprandi G, Alesina R, Ariano R, et al. Characteristics of patients with allergic polysensitization: the polismail study. *Eur Ann Allergy Clin Immunol* 2008;40:77-83.
22. Ciprandi G, Contini P, Fenoglio D, Sormani MP, Negrini S, Puppo F, Indiveri F. Relationship between soluble HLA-G and HLA-A,-B,-C serum levels and IFN-gamma production after sublingual immunotherapy in patients with allergic rhinitis. *Human Immunology* 2008;69:510-2.
23. Ciprandi G, Colombo BM, Contini P, Cagnati P, Pistorio A, Puppo P, Murdaca P. Soluble HLA-G and HLA-A,-B,-C serum levels in patients with allergic

- rhinitis. *Allergy* 2008;63:1335-8.
24. Gelardi M, Maselli del Giudice A, Candreva T, et al. Nasal resistance and allergic inflammation depend on allergen type. *Int Arch Allergy Immunol* 2006;141:384-9.
25. Ciprandi G, Cirillo I, Pistorio A. Persistent allergic rhinitis includes different pathophysiological types. *Laryngoscope* 2008;118:385-8.
26. Fenoglio D, Ferrera A, Ferrera F, Sormani MP, Puppo F, Di Gioacchino M, Ciprandi G. Patients with allergic rhinitis show an allergen-specific interferon-gamma defect. *Eur J Inflamm* 2008;6:87-91.

PROOF