

ALLERGEN-SPECIFIC Ig CLASSES IN NON-ALLERGIC INDIVIDUALSG. CIPRANDI, M. DE AMICI¹, M. TOSCA² and G. MARSEGLIA¹

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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. However, there is no study that has explored the pattern of allergen-specific Immunoglobulin (Ig) classes in normal subjects. Therefore, the aim of this study is to investigate the allergen-specific IgE, IgG, IgG4, and IgA serum level pattern in a group of non-allergic individuals. Forty healthy non-allergic subjects were enrolled. Serum allergen-specific IgE, IgG, IgG4, and IgA for mites, cat, Parietaria, grasses, and birch were quantitatively determined by ELISA method. Allergen-specific IgE, IgG, IgG4, and IgA serum levels were significantly different for each tested allergen ($p=0.0001$ for each class). In conclusion, the present study provides the first evidence that immunoglobulin production pattern depends on the specificity of the allergenic response in non-allergic subjects as well as in allergic patients. In addition, this study is the first that quantitatively evaluates the Ig classes.

Allergic disorders represent the most common immune-mediated diseases, and their prevalence is increasing worldwide (1). Allergic reaction 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 pathogenic phenomenon occurring in allergic patients. In fact, the *stigmata* of allergic reaction is actually the production of specific IgE toward

inhalant allergens. Furthermore, a number of recent studies have indicated that regulatory T cells (Tregs) may play an important role in controlling the Th2-biased responses in allergic patients (3-6). Indeed, the non-allergic response to allergens is characterized by suppression of allergen-specific Th2 responses by functionally active T regulatory cells. This issue appears crucial, as a functional defect of T regulatory cells has been demonstrated by an experimental study on allergic subjects (7). 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

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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 (8). In addition, TGF- β drives the production of IgA (9). The response to allergens in non-allergic subjects and allergic patients should substantially consist of the class pattern of allergen-specific immunoglobulins: such as IgG and IgA in non-allergic subjects, and IgE in allergic patients.

In this regard, a recent study investigated the pattern of allergen-specific IgE, IgG (total and IgG4), and IgA production with relation to the allergen specificity in a group of polysensitized patients (10). This study evidenced that the production pattern of Ig classes is allergen-specific in allergic patients (10).

However, there is no study that has quantitatively explored the pattern of Ig classes specific for the most common allergens in non-allergic subjects. Therefore, the aim of this study is to investigate the allergen-specific IgE, IgG, IgG4, and IgA serum level pattern in non-allergic subjects.

MATERIALS AND METHODS

Study design

The study evaluated 40 non-allergic individuals (20 females, 20 males) with a mean age of 21 years. The skin prick test and blood sampling for assessing serum immunoglobulin levels were performed on all subjects. All subjects had to have a negative skin prick test for a panel of common aeroallergens to be included in the study, according to validated criteria (11). Exclusion criteria included: a diagnosis of allergic disorders, including rhinitis, asthma, conjunctivitis, drug and food allergy, and atopic dermatitis, and also any other acute or chronic disorder, presence of cat at home and living in a rural area. The study was performed between mid-July and mid-August, which is a period with a very low concentration of pollen and mites in order to have the lowest allergen pressure. The study was approved by the local Ethics Committee and written informed consent was given by all participants.

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). Allergen-specific Ig classes were tested for the following allergens: house dust mites, cat, grasses, *Parietaria*, and birch.

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 response 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 (1 kU/l = 0.92 μ g/l). The detection limit is <0.35 kU/l. Values $\geq$ 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 in a single laboratory session (Phadia AB, Uppsala, Sweden) using an IgG or IgG4 ImmunoCAP FEIA assay described previously (10, 13). Briefly, the method consists 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/l; Specific IgG4: 0-300 μ g/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 is <0.02 mg/L. Limit of quantitation for antigen specific IgG4 antibodies is 0.07 mg/L.

Specific IgA

Specific IgA ImmunoCAP (Phadia) were experimentally used for research purposes only (measured for those allergens, using the Fluorenszymeimmunoassay (FEIA), as previously reported (10). The calibrators are traceable to the International Reference Preparation (IRP) 67/86 for Human Serum Immunoglobulins A, G and M from the World Health Organization (WHO). The measuring range for a diluted sample is 1.0-100 mg/l (where A represents antigen-specific antibodies). The detection limit is <0.01 mg/l.

Statistical analysis

Descriptive statistics were first performed and quantitative parameters were reported as median (md) with first and third quartiles. The non-parametric Wilcoxon test was used to compare samples. The non-parametric Kruskal-Wallis rank test was performed to evaluated the analysis of variance between groups of allergens. All tests were two sided and a p-value less than 0.05 was considered statistically significant. The package "MedCalc 9" (Frank Schoonjans, BE) was used for all the analyses.

RESULTS

Not all subjects were sensitised.

Fig. 1 shows that the sum of the measured allergen-specific Ig classes is significantly dependent on the singular class ($p<0.0001$). Moreover, IgG shows the highest concentration, whereas 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: 0.02 kU/L) and cat (md: 0.01 kU/L) (Wilcoxon test; $p<0.0001$), between Mites and Grasses (md: 0.01 kU/L) (Wilcoxon test; $p=0.018$), Mites and Birch (md: 0 kU/L) (Wilcoxon test; $p<0.0001$), Mites and Parietaria (md: 0.03 kU/L) (Wilcoxon test; $p=0.0029$), between cat and Grasses (Wilcoxon test; $p=0.037$), cat and Birch (Wilcoxon test;

$p=0.05$), between cat and Parietaria (Wilcoxon test; $p<0.0001$), between Grasses and Birch (Wilcoxon test; $p<0.0001$), Grasses and Parietaria (Wilcoxon test; $p<0.0001$) and between Birch and Parietaria (Wilcoxon test; $p<0.0001$).

Specific IgA levels significantly changed (Kruskal-Wallis test; $p<0.0001$) among the groups of allergens (Fig. 2); in particular, there was a significant difference of IgA levels between Mites (md: 1.71 mg/L) and cat (md: 15.1 mg/L) (Wilcoxon test; $p<0.0001$), between Mites and Grasses (md: 0.78 mg/L) (Wilcoxon test; $p<0.0001$), Mites and Birch (md: 0.85 mg/L) (Wilcoxon test; $p<0.0001$), Mites and Parietaria (md: 1.06 mg/L) (Wilcoxon test; $p=0.0015$), between cat and Grasses (Wilcoxon test; $p<0.0001$), cat and Birch (Wilcoxon test; $p<0.0001$), between cat and Parietaria (Wilcoxon test; $p<0.0001$), between Grasses and Parietaria (Wilcoxon test; $p=0.0028$) and between Birch and Parietaria (Wilcoxon test; $p=0.02$).

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: 8.87 mg/L) and cat (md: 3.62 mg/L) (Wilcoxon test; $p<0.0001$), between Mites and Grasses (md: 3.36 mg/L) (Wilcoxon test; $p<0.0001$), between Mites and Birch (md: 2.38 mg/L) (Wilcoxon test; $p<0.0001$), between Mites and Parietaria (md: 4.48 mg/L) (Wilcoxon test; $p=0.0001$), between cat and Birch (Wilcoxon test; $p=0.024$), between Grasses and Parietaria (Wilcoxon test; $p=0.033$) and between Parietaria and Birch (Wilcoxon test; $p=0.001$) (Fig. 2).

Specific IgG4 levels significantly changed (Kruskal-Wallis test; $p<0.0001$) among the groups of allergens; in particular, there was a significant difference of IgG4 levels between Mites (md: 0.26 mg/L) and Grasses (md: 0.05 mg/L) (Wilcoxon test; $p<0.0001$), between Mites and Birch (md: 0.01 mg/L) (Wilcox test; $p<0.0001$), between cat (md: 0.27 mg/L) and Birch (Wilcox test; $p<0.0001$), cat and Grasses (Wilcox test; $p<0.0001$), Grasses and Parietaria (md: 0.22 mg/L) (Wilcoxon test; $p=0.0003$), between Parietaria and Birch (Wilcoxon test; $p<0.0001$) and between Grasses and Birch (Wilcox test; $p=0.005$) (Fig. 2).

DISCUSSION

Few studies have investigated the production of

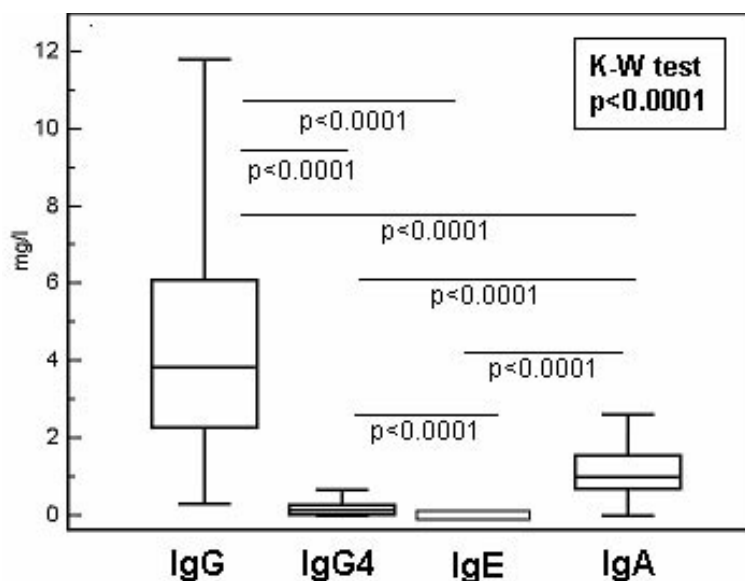


Fig. 1. Ig class (IgE, IgA, IgG, IgG4) distribution in subjects without allergen distinction. Values are represented as median (black line), quartiles (25th and 75th percentiles, black box), lowest and highest values, and p-values between the groups.

allergen-specific Ig classes in non-allergic subjects. A first study reported that the non-allergic response to Der p1 is characterized by specific serum IgA and IgG4, but not IgE or IgG1 (8). Another study reported that exposure to cat allergen can induce an IgG and IgG4 response without sensitization or risk of asthma in children (14). A further study reported that immune responses to birch can be demonstrated in children during the first 7 years of life, regardless of atopic status, and a transient early Th2-like response is down-regulated after the fourth pollen season except in children who developed clinical allergy to birch (15). Hesselmar showed that cat-keeping was associated with a modified Th2 response, producing IgG4, but not IgE antibodies; this immune response was not associated with an increased risk of asthma or allergy, however the IgG4 antibodies did not directly mediate any protective effect (16). The MAS study reported that the IgG levels at school-age correlated with cat allergen exposure during the first 2 years of life and a large percentage of very highly exposed children showed high IgG, but not IgE responses to cat, however, not all the highly exposed children were found to be protected from sensitizations (17). Moreover, it is to note that all these studies measured Ig serum levels using arbitrary units, such

as in non-quantitative manner, whereas this study was designed also to evaluate Ig classes in normal subjects using a quantitative method.

This study globally shows that the response to some allergen sources may induce different Ig class patterns in non-allergic subjects, confirming the previous studies. Analysing the single Ig classes, some interesting findings may be observed: i) regarding IgG, mites induce the highest levels followed by Parietaria, ii) regarding IgG4, mites and cat exert the same effect, iii) regarding IgA, cat allergen induces a very relevant production, and iv) regarding IgE, Parietaria is the most important inducer.

These findings might depend at least on two factors. Firstly, the allergenic property, such as the capacity of inducing and stimulating IgE synthesis, is specific for each allergen. Secondly, the duration of allergen exposure: maximal for mites and cat, followed by Parietaria. In fact, a prolonged exposure to an allergen may amplify the specific immune response. The most relevant and original finding of this study is that allergen-specific Immunoglobulin pattern is allergen-dependent in non-allergic subjects as well as in reported allergic patients. On the other hand, the specificity of the immune response to

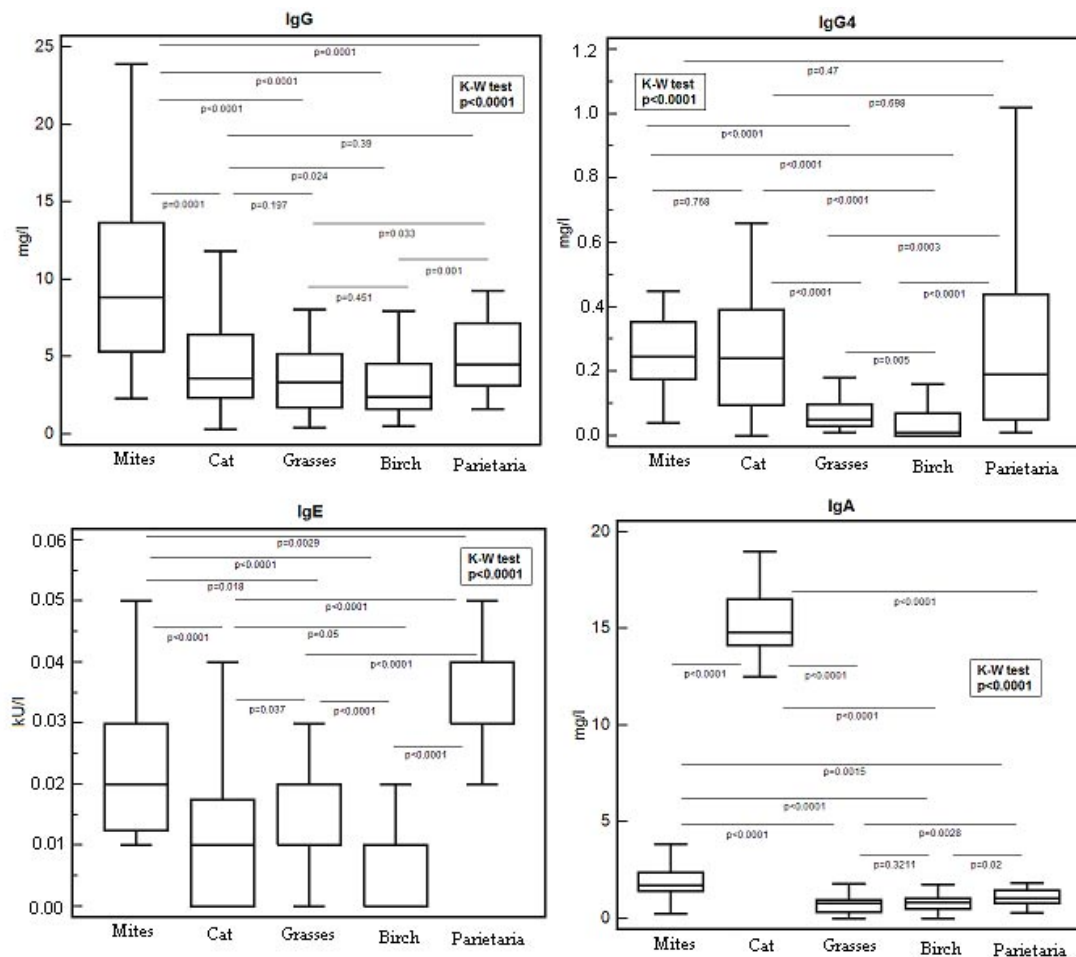


Fig. 2. Serum allergen specific IgE, IgA, total IgG, and IgG4 distribution in subjects evaluated for the single allergens. Values are represented as medians (black line), quartiles (25th and 75th percentiles, black box), lowest and highest values, and p-values between the groups.

allergens has been previously reported as both inflammatory degree and nasal airflow depended on the specific causal allergen (18). In addition, pollen-induced rhinitis is characterized by a more impaired nasal airflow than a mites-induced one (19). It has also been demonstrated that the *in vitro* production of IFN- γ depends on the specific allergen used as stimulus (20) and IL-12 levels depend on particular features (21). Thus, all these studies provide clear evidence that each allergen source induces a specific Ig response.

In conclusion, the present study demonstrates that the immunoglobulin pattern is allergen-specific in healthy subjects. Therefore, these results may be

considered clinically relevant proof of the specificity of immune response to allergens also in non-allergic subjects as the Ig response pattern depends on the specific allergen. These preliminary findings need to be confirmed by further studies conducted on a larger number of subjects and also considering the natural exposure to more relevant quantity of allergens.

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