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IMMUNOGLOBULIN PRODUCTION PATTERN IN ALLERGIC AND NON-ALLERGIC SUBJECTS

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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 (Ig) production in allergic patients. However, no studies have compared the pattern of inhalant allergen-specific Ig classes between allergic patients and normal subjects. The aim of this study is to therefore investigate the allergen-specific IgE, IgG, IgG4, and IgA serum level pattern in a group of patients with pollen allergy and in non-allergic healthy subjects. One hundred and two allergic patients (evaluated both out of and in the pollen season) were enrolled. In addition, 50 healthy non-allergic subjects were recruited during the whole year. Serum allergen-specific IgE, IgG, IgG4, and IgA for Parietaria, grasses, and birch were quantitatively determined by the ImmunoCAP System method. Allergen-specific IgE, IgG, IgG4, and IgA serum levels were significantly different for each tested allergen (p=0.0001 for each class) among groups. Allergic patients, mainly during pollen season, showed the highest IgG, IgG4, and IgA levels. The present study therefore provides the preliminary evidence that Ig production pattern toward inhalant allergens may depend on the specificity of the allergenic response both in non-allergic subjects and allergic patients Allergic patients do not show a defect of IgG and IgA classes. In addition, this study is the first that quantitatively evaluates the Ig classes. However, further studies should include non-allergic subjects evaluated both during and out of the pollen season.

Allergic rhinitis (AR) is sustained by an IgE-dependent inflammation, which displays a predominant interleukin (IL)-4 production by Th2 cells over interferon (IFN)-γ expression by Th1 cells: the so-called Th2 polarization (1-2). 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. Regulatory T cells (Tregs) may also play an important role in controlling the Th2-biased responses in allergic patients (3-6). Indeed, the Th2 response to allergens is suppressed by functionally active T regulatory cells in healthy subjects. This issue appears crucial, as a functional defect of T regulatory cells has been demonstrated by an elegant

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experimental study in allergic subjects (7). In this regard, the production of inhibitory cytokines, such as IL-10 and TGF-β, is relevant for the function of T regulatory cells. IL-10 increases allergen-specific IgG4 production, inhibiting IgE synthesis, and TGF-β drives the production of IgA (8-9). The different response to allergens, existing between normal subjects and allergic patients, should substantially consist in the class pattern of allergen-specific immunoglobulins: such as IgG and IgA in normal subjects, 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 AR patients (10). This study demonstrates that the production pattern of Ig classes is allergen-specific in allergic patients (10).

However, there is no study that has quantitatively compared the pattern of these Ig classes specific for the most common allergens between allergic patients and normal subjects. Therefore, the aim of this study is to investigate the inhalant allergen-specific IgE, IgG, IgG4, and IgA serum level pattern in a group of patients with pollen allergy and in non-allergic healthy subjects.

MATERIALS AND METHODS

Study design

The study evaluated 50 healthy subjects (25 females) with mean age of 22 years, and 102 allergic patients (53 females), evaluated out of and in the pollen season, with a mean age of 39 years. The skin prick test and blood sampling for assessing serum immunoglobulin levels were performed in all subjects.

Healthy subjects had to be negative to skin prick test and without any allergic disorders, including rhinitis, asthma, conjunctivitis, drug and food allergy, and atopic dermatitis, and also any other acute or chronic disorder.

Allergic patients had to have a diagnosis of allergic rhinitis to pollens made according to validated criteria (11), but they had to be without any other acute or chronic disorder. Allergic patients were evaluated outside the pollen season, such as during the mid-winter, and between mid-April and mid-June, which as the period with the highest concentrations of pollen in our geographic area. Healthy subjects were recruited and evaluated during the whole year to avoid the influence of the seasonal allergen exposure on immune response.

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: grasses, Parietaria, and birch.

Specific IgE

Allergen-specific IgE ImmunoCAP (Phadia) was measured using the Fluorenzyme-immunoassay (FEIA). The allergen of interest, covalently coupled to ImmunoCAP, reacts with the specific IgE in the serum specimen. To evaluate the test results, the response for the patient samples are transformed into concentrations with the use of a calibration curve (0-100 kU/l).

ImmunoCAP Specific IgE Calibrators are used for determinating 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 in a single laboratory session (Phadia AB, Uppsala, Sweden) using an IgG or IgG4 ImmunoCAP FEIA assay, as previously described in detail (10, 13). To evaluate the test results, the response for the patient samples were transformed into concentrations with the use of a calibration curve (Specific IgG: 1:100 diluted sample 2-200 mg/l; Specific IgG4: 0-300 μg/l; where A represents antigen-specific antibodies). 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 were <0.02 mg/L. Limit of quantitation for antigen specific IgG4 antibodies is 0.07 μg/L.

Specific IgA

Specific IgA ImmunoCAP (Phadia) were experimentally used for research propose only (measured for those allergens, using the Fluorenzymeimmunoassay (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. 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 evaluate 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

Specific IgE levels significantly changed (Kruskal-Wallis test; $p<0.0001$) among the groups of subjects when we considered all allergens separately (Fig. 1). Table I A reports the serum levels of IgE in the three groups of subjects evaluated separately for birch, grasses and parietaria.

Specific IgA levels significantly changed among the groups of subjects when we considered separately birch (Kruskal-Wallis test; $p<0.0001$), grasses (Kruskal-Wallis test; $p<0.0001$) and parietaria (Kruskal-Wallis test; $p=0.0001$) (Fig. 2). Table I B reports the serum levels of IgA in the three groups of subjects evaluated separately for birch, grasses and parietaria.

Specific IgG levels significantly changed among the groups of subjects when we considered separately birch (Kruskal-Wallis test; $p=0.0031$), grasses (Kruskal-Wallis test; $p<0.0001$) and parietaria (Kruskal-Wallis test; $p=0.0001$) (Fig. 3). Table I C reports the serum levels of IgG in the three groups of subjects evaluated

Table I. *Serum levels of **A)** IgE [kU/L], **(B)** IgA [mg/L], **C)** IgG [mg/l] and IgG4 [mg/L] **(D)**, evaluated separately for group of subjects and allergens. Data are expressed as median with first and third quartile.*

A

	IgE [kU/L] median (25°th-75°th)		
	Birch	**Grasses**	**Parietaria**
Healthy subjects	0.00 (0.00-0.01)	0.01 (0.01-0.02)	0.03 (0.03-0.04)
SAR out season	6.96 (1.2-14.6)	2.45 (0.26-8.19)	50.05 (16.3-94.3)
SAR in season	7.43 (2.4-18.6)	12.9 (2.4-24.1)	51.7 (25.6-73.2)

B

	IgA [mg/L] median (25°th-75°th)		
	Birch	**Grasses**	**Parietaria**
Healthy subjects	0.85 (0.5-1.04)	0.78 (0.34-0.98)	1.06 (0.81-1.48)
SAR out season	0.97 (0.66-1.56)	1.16 (0.9-1.44)	2.2 (1.67-2.72)
SAR in season	2.94 (1.6-3.44)	1.54 (0.93-2.83)	0.96 (0.45-1.32)

C

	IgG [mg/L] median (25°th-75°th)		
	Birch	**Grasses**	**Parietaria**
Healthy subjects	2.38 (1.6-4.55)	3.36 (1.75-5.2)	4.48 (3.15-7.14)
SAR out season	4.87 (3.54-6.21)	2.34 (0.01-2.45)	5.06 (3.88-5.59)
SAR in season	3.75 (2.91-4.95)	8.04 (5.29-8.88)	16.9 (12.65-19.7)

D

	IgG4 [mg/L] median (25°th-75°th)		
	Birch	**Grasses**	**Parietaria**
Healthy subjects	0.01 (0.001-0.07)	0.05 (0.03-0.1)	0.22 (0.05-0.5)
SAR out season	0.26 (0.09-1.04)	0.08 (0.05-0.13)	0.38 (0.3-0.79)
SAR in season	0.16 (0.07-0.46)	0.12 (0.07-0.25)	0.65 (0.42-1.27)

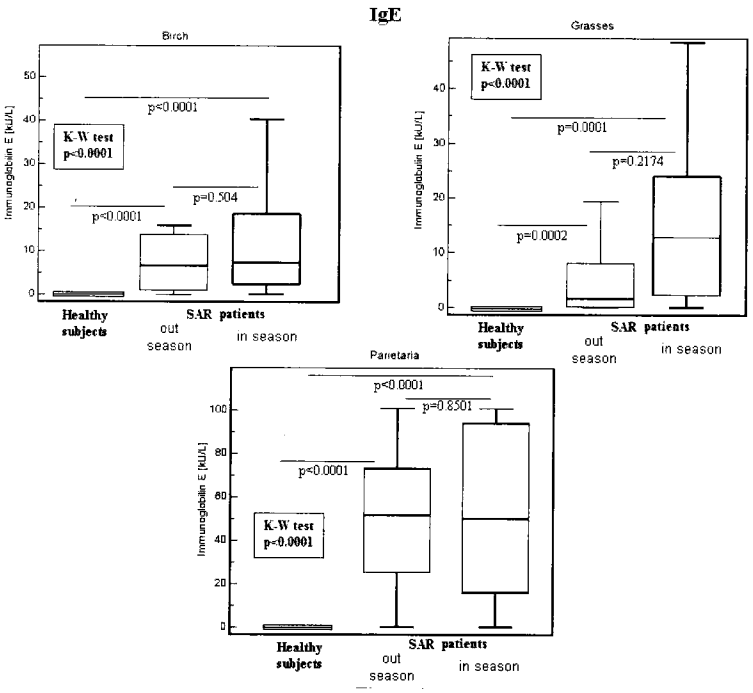

Fig. 1. *Serum allergen specific IgE distribution in subjects evaluated for the single allergens. Values are represented as medians (black line), quartiles (25°th and 75°th percentiles, black box), lowest and highest values, and p-values between the groups.*

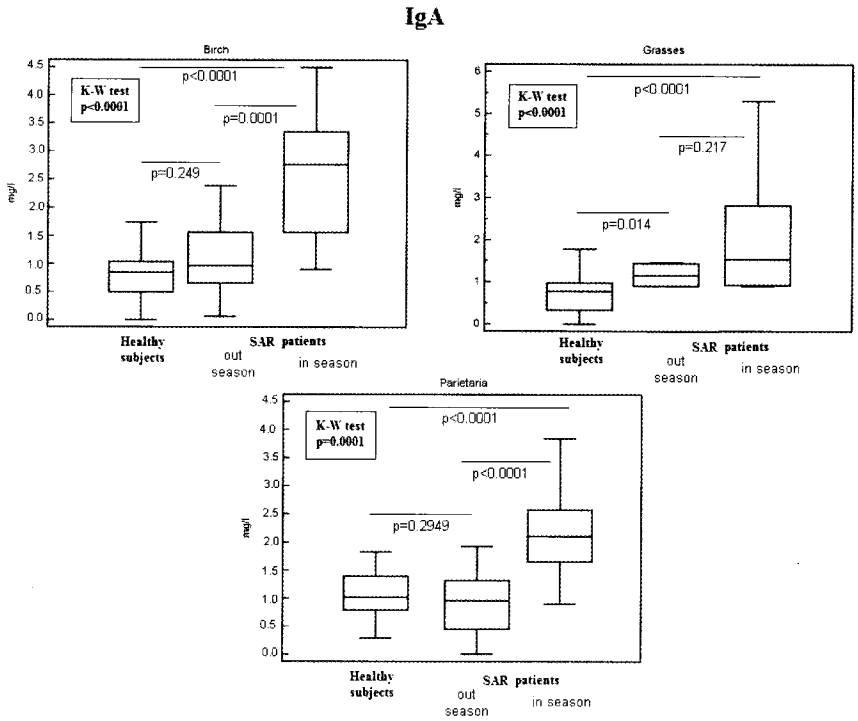

Fig. 2. *Serum allergen specific IgA distribution in subjects evaluated for the single allergens. Values are represented as medians (black line), quartiles (25°th and 75°th percentiles, black box), lowest and highest values, and p-values between the groups.*

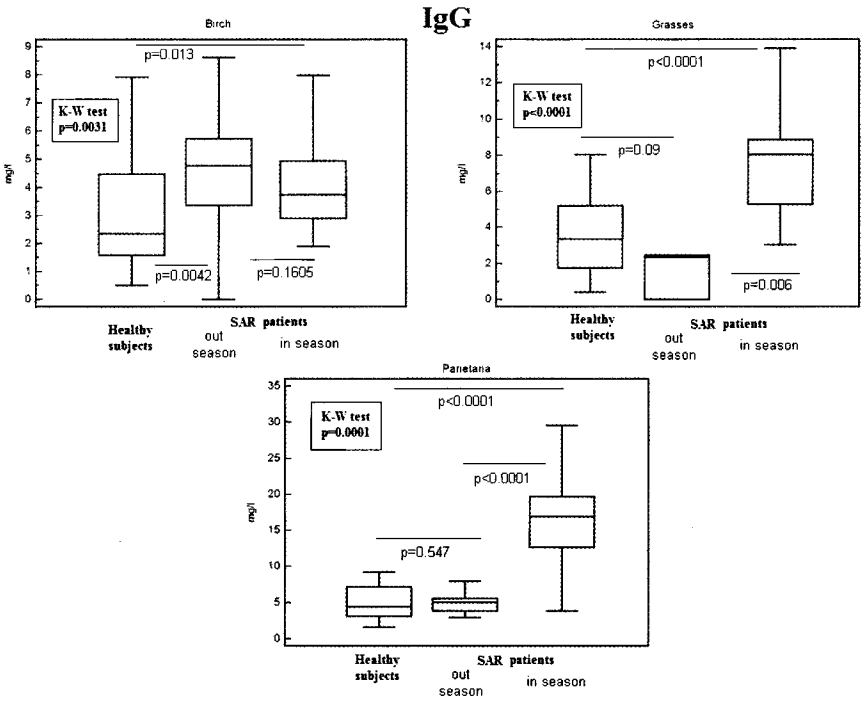

Fig. 3. *Serum allergen specific IgG distribution in subjects evaluated for the single allergens. Values are represented as medians (black line), quartiles (25°th and 75°th percentiles, black box), lowest and highest values, and p-values between the groups.*

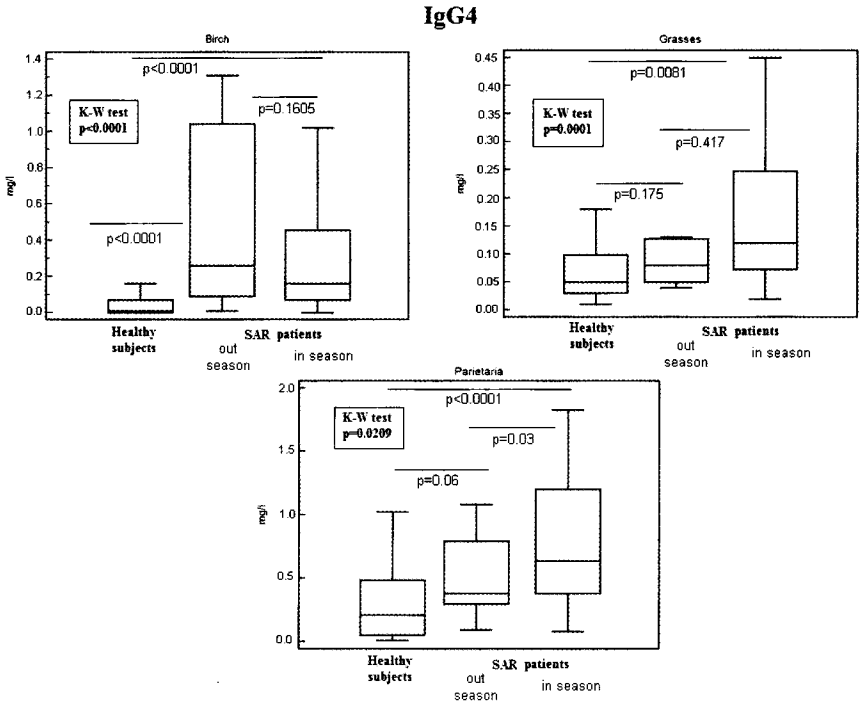

Fig. 4. *Serum allergen specific IgG4 distribution in subjects evaluated for the single allergens. Values are represented as medians (black line), quartiles (25°th and 75°th percentiles, black box), lowest and highest values, and p-values between the groups.*

separately for birch, grasses and parietaria.

Specific IgG4 levels significantly changed among the groups of subjects when we considered separately birch (Kruskal-Wallis test; $p<0.0001$), grasses (Kruskal-Wallis test; $p=0.0001$) and parietaria (Kruskal-Wallis test; $p=0.0209$) (Fig. 4). Table I D reports the serum levels of IgG4 in the three groups of subjects evaluated separately for birch, grasses and parietaria.

DISCUSSION

The type of response to allergens is different between allergic patients and normal subjects as it resides in the different Immunoglobulin class pattern, namely: IgG and IgA in normal subjects and IgE in allergic patients (8). The reason for this difference could be based on a functional defect of T regulatory cells that naturally direct towards a physiological IgG-IgA response. Indeed, allergic patients have a functional defect of allergen-specific Tregs and a polarized Th2 response with ongoing IgE synthesis (7). However, there are few studies that investigated the production of allergen-specific Ig classes for inhalant allergens in normal subjects. Most of them concerned allergic patients and were conducted as trial of immunotherapy: the findings are inconsistent between them (14-17). However, it is to note that all these studies measured Ig serum levels using arbitrary units, such as in a non-quantitative manner.

This study was therefore designed to compare allergen-specific Ig classes between healthy subjects and allergic patients, evaluated both out of and during the pollen season, using a quantitative method.

Firstly, this study globally shows that the response to allergens induces different levels of Ig classes, depending on: the specific allergen, healthy or allergic response, and allergen exposure.

Analysing IgE, healthy subjects had of course the lowest levels (namely <0.35 kU/L), parietaria pollen was capable of inducing the highest levels, followed by grasses and birch. Interestingly, there was no significant effect of pollen season on IgE levels: this finding might partially depend on the particular climate of Liguria, typical Mediterranean area, characterized by mild winter and cool summer, which could favour prolonged pollination.

With regard to IgG, allergic patients showed higher concentrations than non-allergic subjects, mainly during the pollen season. Similar findings were observed both for IgG4 and IgA. These data might support the hypothesis that allergen exposure in allergic patients stimulates the production of IgG and IgA antibodies. A very recent study may confirm these findings: indeed, it has been demonstrated that TGF-β serum levels depends on the level of allergen exposure (18). 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 Parietaria. In fact, a prolonged exposure to an allergen might amplify a persistent specific immune response. Partially, this finding was detected also for other Ig classes: a clear explanation for this phenomenon is obscure. Further studies are needed to address this issue.

The most relevant and original finding of this study is represented by the fact that it provides the first evidence which shows that allergen-specific immunoglobulin pattern production is allergen-dependent in healthy subjects as well as in allergic patients as the levels of Ig were different for each single allergen.. There are allergens more inducive of an Ig response and Tregs function might depend on the type of allergen and the exposure duration. This study therefore evidences that each single allergen is able of inducing a distinct immune and clinical response.

In conclusion, the present study demonstrates that the immunoglobulin pattern is allergen-specific and may partially depend on the level of allergen exposure. In addition, allergic patients do not show a defect of IgG and IgA classes in comparison with healthy subjects. However, these preliminary findings need to be confirmed by further studies conducted on a greater number of subjects and evaluating non-allergic subjects both in and out of the pollen season.

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