ORIGINAL CONTRIBUTION

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Diminished response to grass pollen allergen challenge in subjects with concurrent house dust mite allergy*

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SUMMARY

Introduction: *The clinical manifestation of allergic rhinitis is influenced by many factors; while different subpopulations are not well defined. Different combinations of allergic sensitization may lead to different clinical manifestations of allergic disease.*

Methods: *In a nasal allergen challenge model we compared allergic rhinitis symptoms between subjects mono-sensitized to grass pollen or house dust mite, poly-sensitized subjects, and healthy controls. We measured visual analogue scales of symptoms and peak nasal inspiratory flow. We also compared serum total IgE, allergen-specific IgE and IgG4, and basophil histamine release.*

Results: *Nasal challenge with grass pollen extract led to a significantly larger increase in subjective ($p = 0.031$) and objective ($p = 0.001$) nasal symptoms in grass pollen mono-sensitized subjects than in poly-sensitized subjects. No differences were found in serum levels of allergen-specific IgE and IgG4 or in biological activity of IgE (basophil histamine release) between mono-sensitized and poly-sensitized subjects. We found a strong inverse correlation between serum allergen-specific IgE and basophil histamine release (-0.789, $p = 0.001$).*

Conclusions: *Grass pollen mono-sensitized subjects have a more severe clinical response to nasal challenge than poly-sensitized subjects. This cannot be explained by serum levels of IgE or its biological activity. The continuous allergen exposure in poly-sensitized subjects may alter local immuno-regulatory processes, leading to a reduced clinical response to allergen challenge.*

Key words: allergic rhinitis, nasal allergen provocation, IgE, IgG4, basophil histamine release, mono-sensitization, poly-sensitization

INTRODUCTION

Allergic disease is a systemic disorder that can present with different clinical manifestations such as allergic rhinitis (AR), asthma or atopic dermatitis. Even within the group of AR, the disease can present in various ways due to differences in the type and number of allergic sensitizations. AR is classified as intermittent or persistent, and as mild or moderate-severe, in accordance with the workgroup document Allergic Rhinitis and its Impact on Asthma (ARIA) [(1)].

Other factors that influence AR symptoms are allergen exposure and the effect of repeated allergen challenges. Daily repeated allergen challenges can lead to an increased responsiveness of the nasal mucosa [(2)]. However, they may also fail to increase the allergic response or even reduce responsiveness [(3-6)]. How the clinical response is reflected in terms of mucosal inflammation is even more complicated. AR is a chronic inflammatory disease, but there is no clear correlation between AR symptoms and nasal mucosal inflammation [(7)]. Even when the sensitizing allergen and symptoms are absent, e.g. in grass pollen allergic subjects out of season, minimal mucosal inflammation may persist [(8)]. It is unclear which inflammatory mechanisms lead to sensitization to an allergen, to symptomatic disease, and to increased or decreased symptomatic responses after prolonged allergen exposure. If we want to investigate

Footnote. Abbreviations: AR: Allergic Rhinitis; BasoHR: Basophil Histamine Release; *Der p*: Dermatophagoides pteronyssinus; GP: Grass Pollen; HDM: House Dust Mite; Monosens-GP: Subjects with mono-sensitization to grass pollen allergen; Monosens-HDM: Subjects with mono-sensitization to house dust mite allergen; NP: Nasal Allergen Provocation: PEF: Peak Expiratory Flow; *Phl p*: Phleum pratense; PNIF: Peak Nasal Inspiratory Flow; Polysens-provGP: Subjects with poly-sensitization who underwent allergen provocation with grass pollen allergen; Polysens-provHDM: Subjects with poly-sensitization who underwent allergen provocation with house dust mite allergen; TNSS: Total Nasal Symptom Score; VAS: Visual Analogue Scale.

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these mechanisms it is very important to define subpopulations of AR subjects. We looked at mono-sensitized and poly-sensitized subjects with grass pollen (GP) and house dust mite (HDM) allergy to compare the effects of nasal allergen challenge.

In a previous study we investigated the effect of desloratadine treatment on AR and asthma [9]. In that study, we found that there was a large variability in response to nasal allergen provocation (NP) after provocation with the same amount of GP extract. Subgroup analysis showed differences between subjects with grass pollen mono-sensitization and subjects with poly-sensitization (relevant grass pollen and other). We found a trend to lower total nasal symptom score in poly-sensitized subjects compared to subjects with grass pollen mono-sensitization, however groups were too small to reach significance. Furthermore, we found more symptom reduction in the poly-sensitized subjects after antihistamine treatment (unpublished data).

In this study we evaluated the effect of NP on clinical parameters in allergic rhinitis subjects with mono-sensitization and poly-sensitization, and the differences in clinical response to NP with grass pollen and house dust mite. We hypothesized that nasal symptoms would be less in poly-sensitized subjects compared to subjects with grass pollen mono-sensitization. To find a possible explanation for the differences in clinical response we measured serum total immunoglobulin E (IgE), allergen-specific IgE, and allergen-specific IgG4. Also, biological activity of IgE was determined by basophil histamine release *in vitro*.

MATERIALS AND METHODS

Subjects

The subjects in this study were adults with seasonal or perennial allergic rhinitis and were compared with healthy controls. The following distinct groups were formed: subjects with seasonal AR symptoms and grass pollen sensitization (Monosens-GP, n = 14); subjects with perennial rhinitis and sensitization to house dust mite without pollen sensitization (Monosens-HDM, n = 9); subjects with perennial rhinitis with increasing symptoms during the pollen season and multiple sensitizations at the skin prick test, including house dust mite and grass pollen (Polysens, n = 29); healthy controls (Controls, n = 14).

The diagnosis of AR was based on a history of rhinitis symptoms for at least two years, and a positive skin prick test to grass pollen and/or house dust mite. At screening, all subjects were tested for a panel of 10 common inhalant allergens (ALK-Abello BV, Nieuwegein, the Netherlands). A skin prick test was considered positive when the wheal diameter was 3 mm larger than that produced by the negative control after 15 minutes. Controls had a negative skin prick test. Subjects with asthma were allowed to participate in the study if they were clinically stable (i.e. no hospital admissions, exacerbations, or respiratory tract infections in the last 4 weeks prior to inclusion) and able to stop their asthma medication during the study. All subjects were non-smokers. Subjects were excluded if they suffered from a disorder likely to interfere with the test results. All medications likely to interfere with the study results were stopped before enrolment, as appropriate (e.g. intranasal, inhaled, or oral steroids 30 days; antihistamines 48 hours; leukotriene inhibitors 10 days). Subjects with prior immunotherapy were excluded.

Table 1. Subject characteristics.

GROUP	AGE (y) Median (range)	SEX (M:F)	Asthma
Monosens-HDM	27 (19-62)	5:4	0/9
Monosens-GP	28 (18-53)	6:8	0/14
Polysens-provHDM	23 (18-59)	3:10	5/13
Polysens-provGP	22 (18-39)	7:9	4/16
Controls	22 (19-52)	4:10	0/14

Study design

This study evaluated the clinical response to a single nasal allergen provocation (NP), comparing subjects with seasonal AR or perennial AR to healthy controls. NP is a commonly accepted method for studying the allergic response [10]. Seventy-one eligible subjects were included in this study. Demographic characteristics were comparable for all groups (Table 1).

Subjects in the Monosens-GP group were challenged with grass pollen extract (grasses mix, ALK-Abello BV), subjects in the Monosens-HDM group were challenged with house dust mite extract (*Dermatophagoides pteronyssinus*, ALK-Abello BV). Subjects in the poly-sensitized group and the healthy controls were randomly challenged with either grass pollen or house dust mite allergen (Figure 1). Subjects were challenged with 10,000 BU/mL grass pollen or house dust mite extract (ALK-Abello) via a pump spray delivering one fixed dose of 89 μL into each nostril.

After NP the following symptoms were recorded on a 100 mm visual analogue scale (VAS): rhinorrhoea, nasal itching, sneezing, nasal blockage, and watery eyes. Visual analogue scales of multiple symptoms are accurate for measuring differences in symptoms [11]. Obstruction of the upper airways was assessed by peak nasal inspiratory flow (PNIF) [12]. PNIF was measured

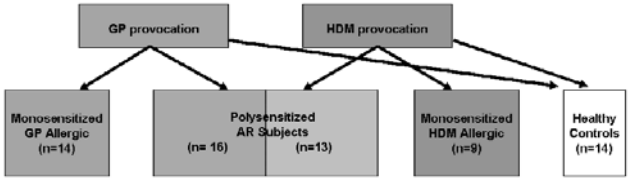

Figure 1. Study design. Mono-sensitized subjects were challenged with the relevant allergen. Poly-sensitized subjects and healthy controls were randomly challenged with either grass pollen or house dust mite allergen.

using an in-check inspiratory flow meter with face mask (Clement Clarke International Ltd., Harlow, England). The highest value of three measurements was recorded. Symptom scores and functional parameters were recorded at baseline, 15-30-45-60-90-120 minutes, and 24 hours after NP.

The study was conducted outside the grass pollen season; recruitment began in October 2004 and was completed in April 2005. All participants gave written informed consent to the study, which was approved by the medical ethics committee of the Academic Medical Center, Amsterdam, the Netherlands under project number MEC03/201.

Determination of IgE and IgG4

Serum IgE and IgG4 specific to *Dermatophagoides pteronyssinus* (d1) and timothy grass (g6), as well as total IgE, were analyzed using the CAP FEIA system (Phadia, Uppsala, Sweden). Blood samples were drawn at baseline, before the allergen challenge.

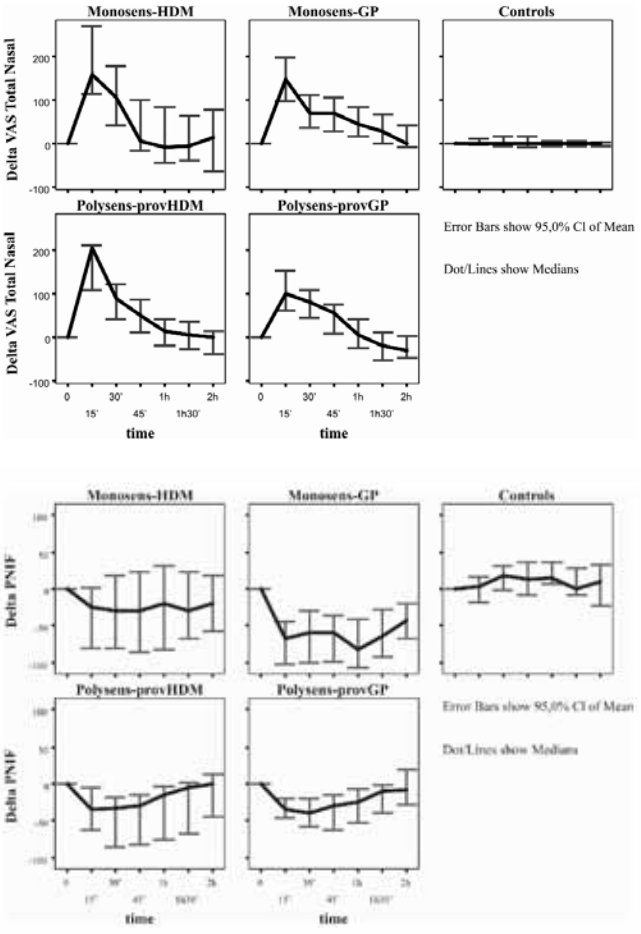

Figure 2. A) Total Nasal Symptom Score (TNSS) in mm. B) Peak Nasal Inspiratory Flow (PNIF) in L/min, during the first 2 hours after allergen provocation (time in minutes). Different kinetics of both Delta VAS and Delta PNIF – symptoms are sustained in the GP-monosensitized group whereas they are transient in the other groups – results in the observed differences in the area under the curve.

Stripped basophil histamine release bioassay

Stripped basophil histamine release bioassay was performed as described earlier [13]. In short, white blood cells were isolated from whole blood of a non-allergic donor by Percoll centrifugation. Basophils were stripped of their IgE by lactic acid treatment (pH 3.9), resensitized by human sera (n = 14) and stimulated with *Phleum pratense* allergen in the range of 0.001 μg/ml to 100 μg/ml. Histamine release was determined by fluorometric analysis, essentially as described by Siraganian [14]. Histamine release was expressed as the percentage of the total amount of histamine in the cells, determined by lysis of the cells with perchloric acid. Stripped cells were used as a negative control and induced a histamine release lower than 3%.

Statistical analysis

All data were analyzed by SPSS software (version 11.5.1). Repeated measurements of nasal symptom scores and PNIF were calculated as area under the curve. We used non-parametric tests to analyze statistical significance. We compared clinical and functional data from multiple groups using the Kruskal-Wallis test, and between two groups using the Mann-Whitney U test. Univariate correlations were calculated with the Spearman rank test. Multivariate analysis was not performed, because the distribution was skewed. A p-value less than 0.05 was considered significant.

RESULTS

Nasal symptom scores and PNIF measurements

Nasal allergen provocation (NP) with a relevant allergen (either GP or HDM extract) led to a significant increase in the total nasal symptom score in all allergic subjects ($p < 0.005$ in all groups; Figure 2). An interesting observation was that the subjects with GP mono-sensitization had a significantly higher ($p = 0.031$) total nasal symptom score (TNSS) than the subjects with perennial AR and poly-sensitization. The maximum TNSS was reached after 15 min, with a median value of 147 in the Monosens-GP group, as compared to 100 in the Polysens-provGP group. The median area under the curve during the first 2 hours after NP was 93.6 mm*h in the Monosens-GP group, as compared to 42.1 mm*h in the Polysens-provGP group ($p = 0.050$). PNIF values, as an objective measurement of nasal patency, supported these observations. We found a significant decrease in PNIF values after NP in all allergic subjects ($p < 0.005$ for all groups). Furthermore, subjects with GP mono-sensitization had significantly lower PNIF values than subjects with perennial AR and poly-sensitization ($p = 0.001$), and their values were also lower than in both groups challenged with house dust mite ($p = 0.001$). After NP with HDM we did not find a significant difference in clinical response – i.e. nasal symptom scores and PNIF – between subjects with mono-sensitization to HDM and subjects who were poly-sensitized.

Total and allergen-specific immunoglobulin E

To determine whether the observed differences in clinical response to GP challenge between mono-sensitized and poly-sensitized subjects could be explained by differences in serum IgE, we measured total and allergen-specific IgE for HDM and timothy grass pollen. High serum levels of IgE lead to higher numbers of FcεRI receptors on mast cells [15,16] and allergen-induced IgE crosslinking on mast cells initiates histamine release [17,18], leading to allergic symptoms.

Serum levels of total IgE, and allergen-specific IgE for *Dermatophagoides pteronyssinus* [*Der p*]*1* and *Phleum pratens*e *[Phl p]* pollen were measured in all subjects, and we calculated the ratio of allergen-specific to total IgE for each study group (Figure 3). Levels of total IgE were highest in the groups with symptomatic allergy and poly-sensitizations (median levels 381.5 and 318 kU/l), followed by the groups with isolated HDM and isolated GP sensitization (median levels 193 and 110.5 kU/l, respectively). We found very low levels of total IgE (median 16.2 kU/l) in the healthy control group. Total IgE was significantly higher in the polysensitized subjects than in the GP-monosensitized subjects ($p < 0.01$), and total IgE was significantly higher in all allergic subjects compared to the healthy controls ($p < 0.001$). High levels of *Der p*-specific IgE were found in symptomatic subjects with positive skin prick tests for HDM (Monosens-HDM, Polysens-provGP, and Polysens-provHDM groups; median levels 10.6, 12.15, and 25.9 kU/l respectively). Timothy grass pollen-specific IgE levels were high in subjects with positive skin prick tests for grass pollen (Monosens-GP, Polysens-provGP, and Polysens-provHDM groups; median levels 9.72, 9.21, and 8.68 kU/l, respectively).

We calculated the ratio of allergen-specific IgE to total IgE. The median ratios of *Der p*-specific IgE to total IgE in the groups Monosens-HDM, Polysens-provGP, and Polysens-provHDM were 0.1, 0.07, and 0.08, respectively. Observed differences were not significant. The median ratio of *Phl p*-specific IgE to total IgE in the Monosens-GP group was 0.14, which was significantly higher than the ratios found for the Polysens-provGP and Polysens-provHDM, i.e. 0.03 and 0.02, respectively ($p = 0.001$ for both groups).

In summary, we did not find significant differences in *Phl p*-specific IgE between the Monosens-GP group and the Polysens-provGP group, nor did the skin prick test show differences in wheal diameter for grass pollen between both groups (data not shown). Furthermore, we did not find any statistically significant correlations between the amount of nasal symptoms measured in the first 2 hours after NP and total IgE, grass pollen-specific IgE, house dust mite-specific IgE, or the ratio of specific to total IgE (data not shown). However, we did find a significant lower ratio of *Phl p*-specific IgE to total IgE in the poly-sensitized subjects compared to the grass pollen mono-sensitized subjects.

Basophil Histamine Release (BasoHR)

To compare the biological activity of GP-specific IgE antibodies in mono- and poly-sensitized patients, BasoHR was performed with *Phl p* extract in a subgroup of patients from the Monosens-GP group ($n = 7$) and the Polysens-provGP group ($n = 7$). Sera from the analyzed subjects were representative for the whole group in terms of ratio *Phl p*-specific IgE to total IgE, which was significantly lower in the Polysens-provGP group ($p = 0.017$). All patients had a positive BasoHR test, with maximum histamine release between 52% and 64% at an allergen concentration of 100 μg/mL. Biological activity of GP-specific IgE was similar for both mono- and poly-sensitized patients ($p > 0.2$). A strong inverse correlation was found between serum levels of GP-specific IgE and the allergen concentration needed for 35% histamine release (-0.789, $p = 0.001$) (Figure 4). The correlation between the ratio of allergen-specific IgE to total IgE, and the allergen concentration needed for 35% histamine release, was weaker (-0.552, $p = 0.041$), suggesting that higher total IgE is unlikely to be the explanation for the clinical difference observed between mono- and poly-sensitized patients.

Allergen-specific IgG4

We found higher levels of *Der p*-specific IgG4 in symptomatic subjects with positive skin prick tests for house dust mite (Monosens-HDM, Polysens-provGP, and Polysens-provHDM groups; median levels 108, 115, and 128 kU/L, respectively) than in healthy controls (median 36.8 kU/L, $p < 0.005$ for all groups) and in the mono-sensitized GP-allergic subjects (median 37.6 kU/L, $p < 0.005$ for all groups). Grass pollen-specific

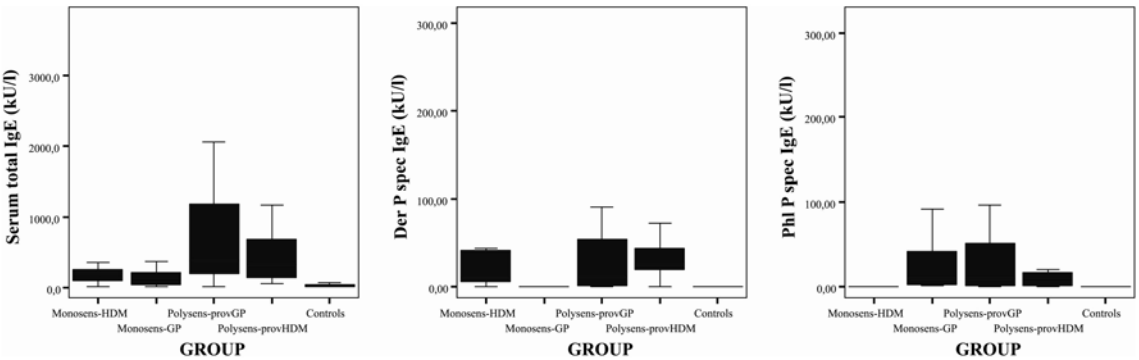

Figure 3. Total IgE (kU/L) and allergen specific IgE (kU/L) in serum for *Dermatophagoides pteronyssinus* and *Phleum pratense.*

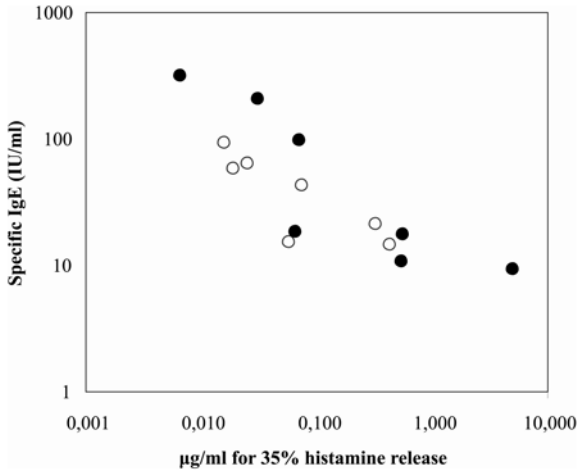

Figure 4. Strong inverse correlation between serum levels of grass pollen specific IgE (IU/mL) and allergen concentration leading to 35% histamine release (Spearman -0.789, p = 0.001). Circled dots = mono-sensitized grass pollen-allergic subjects. Black dots = poly-sensitized subjects.

IgG4 levels were higher in subjects with positive skin prick tests for grass pollen (Monosens-GP, Polysens-provGP, and Polysens-provHDM groups; median levels 82.7, 59.2, and 35.4 kU/l, respectively) than in healthy controls (median 24.8 kU/L) and mono-sensitized HDM-allergic subjects (median 26.8 kU/L), but these differences did not reach statistical significance. *Phl p*-specific IgE and IgG4 were significantly correlated in the Monosens-GP group (0.738, p = 0.003) and in the Polysens-provGP group (0.586, p = 0.017). The levels of *Phl p*-specific IgG4 in serum of the Monosens-GP group were higher than in serum of the Polysens-provGP group, but this difference was not statistically significant and this cannot explain the difference in clinical response between these groups.

DISCUSSION

The aim of this study was to investigate the clinical response to nasal allergen challenge in subjects with grass pollen and house dust mite allergy and the difference between subjects with mono-sensitization and poly-sensitization. The main conclusion from our data is that subjects with grass pollen mono-sensitization had a significantly higher total nasal symptom score and a greater decrease in nasal patency after grass pollen allergen challenge with the same dose of grass pollen allergen than subjects with poly-sensitization. We did not find any significant differences in clinical response to house dust mite challenge between subjects with house dust mite mono-sensitization compared to subjects with poly-sensitization. These data suggest that continuous allergen exposure in concurrent HDM allergy may lead to a decreased responsiveness to GP allergen challenge. This clinical phenomenon may also influence the response to treatment, and therefore has implications for inclusion of allergic rhinitis subjects in clinical trials. It is unclear which mechanism can cause this effect.

The effect of allergen exposure on the immune system is still a matter of debate. Daily repeated allergen challenges lead to increased responsiveness in the nasal mucosa, known as the priming effect [2]. Connell described that this phenomenon was non-specific; meaning that after repeated allergen challenge with one allergen the subject could be primed to a second allergen to which he was known to be allergic. However, a number of studies later showed that repeated allergen challenge might leave the allergic response unaltered or even result in decreased responsiveness [3-5,19]. Furthermore, the response to repeated allergen challenge may change in different circumstances, depending on the number of and the interval between repeated challenges. Some subjects have a natural potential to down-regulate the allergic response after repeated exposure, which may also explain the natural course of the disease: decreasing allergic symptoms with age [20-22]. In some subjects, down-regulation may be induced by the administration of high doses of allergen during immunotherapy [6,23,24]. We conclude from our data that natural exposure to perennial allergens, such as house dust mite, can lead to a reduced response to grass pollen allergen. The higher response to GP challenge in GP-monosensitized subjects was not due to priming by GP, because all subjects were studied outside the GP season, and all underwent a single nasal allergen challenge. Whether these findings translate to real clinical disease during a pollen season is however unknown.

The reduced responsiveness to grass pollen allergen challenge in poly-sensitized subjects was not simply due to lower serum levels of *Phl p*-specific IgE. The relationship between the level of allergen-specific IgE antibodies and the clinical expression of allergy has previously been described by Pastorello et al. [25]. Nasal symptoms are provoked by histamine release from mast cells and basophils after crosslinking of two allergen-specific IgE molecules on the cell surface. The crosslinking of IgE that is bound to the high affinity receptor FcεRI results in the secretion of pro-inflammatory mediators. This response can be enhanced markedly in cells that have been exposed to high serum levels of IgE [16,26-28]. However, we did not find a significant difference in the serum levels of total or allergen-specific IgE between GP-mono-sensitized and poly-sensitized subjects. Moreover, the basophil histamine release bioassay showed that histamine release is determined by allergen-specific IgE and is not affected by the serum level of total IgE or the ratio of allergen-specific IgE to total IgE. Although the median ratio of *Phl p*-specific IgE to total IgE in the group with poly-sensitization was significantly lower than the ratio in the group with grass pollen mono-sensitization, histamine release was equal in both groups. This contradicts the hypothesis that high titers of total IgE may saturate FcεRI, thereby preventing sufficient occupancy of receptors with allergen-specific IgE [29]. Our findings are consistent with MacGlashan, who has previously shown that the density of unoccupied FcεRI on human basophils is remarkably constant across a large range of total receptor densities [30].

We found no significant differences in the serum levels of *Phl p*-specific IgG4 between the mono-sensitized and the poly-sensitized subjects. IgG4 antibodies are thought to antagonize or block the allergic inflammation cascade resulting from allergen recognition by IgE [31]. However, only a few studies on this topic have been performed and data are contradictory. The less severe clinical response in poly-sensitized subjects in this study cannot be explained by a shift in balance between IgE and IgG4. As a matter of fact, there was a trend for the levels of specific IgG4 to be higher in mono-sensitized subjects than in poly-sensitized subjects. We found a significant correlation between specific IgG4 and specific IgE levels. A protective role for IgG4 as an explanation for the observed difference between mono- and poly-sensitized patients is therefore highly unlikely.

We hypothesize that local immuno-regulatory processes affect responsiveness to allergens. Continuous allergen exposure in poly-sensitized subjects may alter the local inflammatory state, possibly in a similar mechanism to that induced by immunotherapy. A difference in immune response between mono-sensitized and poly-sensitized subjects was also found by Rudin et al. [32]. They concluded that T cells specific to seasonal allergens circulate in the blood out of season only if the child is concomitantly sensitized to a perennial allergen. In our study we found a more severe clinical response in grass pollen mono-sensitized subjects. Analogous to the conclusion by Rudin et al., we might hypothesize that regulatory T cells specific to seasonal allergens circulate in the blood out of season only if the subject is concomitantly sensitized to a perennial allergen.

Another explanation could be cross-suppression. *In vitro* studies have demonstrated that CD4+CD25+ regulatory T cells specific for one peptide/MHC complex can suppress the response of CD4+CD25- effector T cells specific for a second peptide/MHC complex. The regulatory T cells require antigen-specific stimulation via the T-cell receptor to become suppressive, but once activated suppressor function is completely antigen non-specific [33]. It is also possible that releasability of basophils may change due to changes in FcεRI signaling pathway molecule expression [34,35]. Further studies are necessary to investigate local factors in the nasal mucosa influencing the response to allergen challenge.

We conclude that subjects mono-sensitized to grass pollen have a more severe clinical response to nasal allergen challenge in terms of symptom scores and nasal patency than subjects with poly-sensitization. Serum levels of total, grass pollen-specific, and house dust mite-specific IgE cannot explain this difference in clinical response. We found a strong correlation between the serum level of *Phl p*-specific IgE and histamine release from basophils, and we did not find differences in histamine release between mono-sensitized and poly-sensitized subjects. We therefore hypothesize that local immuno-regulatory processes affect the responsiveness to allergens. Continuous exposure to house dust mite in poly-sensitized subjects may alter the local inflammatory state, possibly in a mechanism similar to that induced by immunotherapy, leading to a reduced clinical response to grass pollen allergen challenge.

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