

The blocking activity of birch pollen-specific immunotherapy-induced IgG4 is not qualitatively superior to that of other IgG subclasses

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Abstract

Allergen-specific IgG antibodies induced by specific immunotherapy (SIT) interfere with the allergen–IgE interaction, and act as blocking antibodies *in vitro*. It has been hypothesised that IgG4, as opposed to other IgG subclasses, is particularly important in this function, which may play a role for the clinical efficacy of SIT.

In this study, fractionated serum samples from 14 SIT-treated birch pollen allergic individuals enabled determination of the inhibitory capacity of IgG4 alone versus non-IgG4 IgG. Allergen-binding activities of IgG and the IgG-mediated inhibition of allergen binding to autologous IgE were detected using ¹²⁵I-labelled rBet v 1.2801, a recombinant variant of the major allergen of *Betula verrucosa* pollen.

Results show that IgG4-depletion resulted in equivalent reductions in binding and blocking activities. In contrast, a significant but less than two-fold higher relative blocking activity was found in the purified IgG4 fraction. There was no significant difference in the binding avidities (1/*K_d*) measured in the two IgG fractions.

Thus, it appears that SIT-induced specific IgG4 contributes to the IgG blocking of allergen binding to IgE in a simple quantitative manner and not by a particular intrinsic blocking activity.

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1. Introduction

Specific immunotherapy (SIT) is a documented potentially curative treatment of IgE-dependent allergy (Bousquet et al., 1998). The immunological mechanisms underlying the beneficial effects of SIT are, however, not entirely clear. One of the earliest recognized effects of SIT is the induction of allergen-specific IgG (Cooke et al., 1935; Lichtenstein et al., 1968). This IgG has been termed “blocking IgG” because of the ability to inhibit passive cutaneous anaphylaxis, inhibit allergen–IgE interaction and thereby basophil histamine release, and the ability to inhibit serum–IgE-facilitated antigen presentation to T-cells (Cooke et al., 1935; Lichtenstein et al., 1968; Leynadier et al., 1986; Pecquet et al., 1989; Witteman et al., 1996; van Neerven et al., 1999).

A current hypothesis of SIT mechanisms involves the induction of IL-10 and TGFβ producing CD4⁺CD25⁺ regulatory T-cells inducing an anergic or tolerant state in peripheral T cells (Jutel et al., 2003; Nouri-Aria et al., 2004; Blaser and

Akdis, 2004). IL-10 may furthermore decrease IL-4-induced IgE switching and enhance IL-4-induced IgG4 switching, but increase IgE production from B cells that are already IgE switched; whereas TGFβ is an IgA isotype switching factor as well as an inhibitor of IgE isotype switching (Jeannin et al., 1998; Cazac and Roes, 2000; Sugai et al., 2003).

The SIT-induced increase in specific IgG reactivity has been found to occur primarily in the IgG4 subclass (Devey et al., 1976; van der Giessen et al., 1976) and particularly IgG4 has been suggested to possess the ability to act as “blocking antibodies” (Aalberse et al., 1983; Van der Zee and Aalberse, 1987). Clinical studies comparing SIT-induced IgG4 and clinical parameters have shown conflicting results. Some studies show a correlation between specific IgG4 and clinical improvement (Ohashi et al., 1987; Nakagawa et al., 1987; Gehlhar et al., 1999) whereas others have found no correlation (Hedlin et al., 1986; McHugh et al., 1990).

In vitro, blocking of allergen-induced histamine release and suppression of allergen binding to IgE has also been found with sera from untreated patients (Lichtenstein et al., 1968; Gleich et al., 1982; Svenson et al., 2003). In addition,

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tion, high avidity-binding of the allergen has been found analysing IgG of both untreated and SIT-treated patients (Adkinson et al., 1979; Svenson et al., 2003).

It is not clear, however, whether there is a relationship between the relative allergen-binding and the relative blocking activity, respectively, of allergen-specific IgG4. In other words it remains to be established whether IgG4 has a particular intrinsic blocking activity, as compared to other IgG subclasses.

In this study, the binding activities of IgG4 and non-IgG4 IgG from SIT-treated patients were compared to the ability to block allergen-binding to autologous IgE. For these purposes, we used sera from SIT-treated birch pollen-allergic patients and a recombinant variant of Bet v 1, Bet v 1.2801, the major allergen of *B. verrucosa* pollen.

2. Materials and methods

2.1. Patients and serum samples

Sera were collected from birch pollen-allergic patients before and after SIT with *B. verrucosa* pollen extract (Alutard SQ, ALK-Abelló A/S, Hørsholm, Denmark) for 30 weeks (five patients) and 5 years (nine patients). Patients and treatment are described in detail in Bodtger et al. (2002) and Svenson et al. (2003), respectively. The serum samples studied here were selected from patients with specific IgE > 10 kUA/l measured by the CAP system (Pharmacia, Uppsala, Sweden), since this was necessary to measure inhibition of allergen-binding to autologous IgE. All patients reported marked improvements in symptoms during and following SIT. Serum samples were collected outside the birch pollen season.

2.2. Radiolabelling of rBet v 1

Recombinant Bet v 1.2801 (EMBL accession number Z80104) was expressed and purified as described (Spangfort et al., 1996).

Recombinant Bet v 1.2801 (MW: 17 kD) was labelled with ^{125}I using chloramine-T and subsequently subjected to molecular size chromatography on Sephadex G-75 superfine columns (Pharmacia) (Svenson et al., 2003). Fractions where more than 95% of ^{125}I -rBet v 1 bound specific IgG and IgE from birch pollen-allergic patients were used for the experiments. This was obtained by periodic chromatographic re-separation.

The specific activity was calculated to $2\text{--}3 \times 10^5$ cpm/ng.

2.3. Fractionation of serum

2.3.1. Preparation of IgE-depleted serum

Serum depletion of IgE was achieved by co-incubating the serum with supraoptimal amounts of anti-IgE monoclonal antibody (ALK-Abelló) covalently coupled to paramagnetic

beads for 5 h at 4 °C (Johansen et al., 1998). IgE removal was verified by co-incubating the IgE-depleted serum samples with anti-IgE beads followed by washing and test for ^{125}I -rBet v1-binding to the beads (Svenson et al., 2003). Residual activity of less than 4% was detected.

2.3.2. Fractionation of IgE-depleted serum

IgE-depleted sera were further fractionated on a column with immobilised anti-human IgG4 monoclonal antibody at 4 °C. Dialysed mouse ascites containing anti-human IgG4 monoclonal antibody G4.T9 (ALK-Abelló) was immobilised on divinyl sulfon-activated agarose beads (Mini-Leak Low, Kem-En-Tec, Copenhagen, Denmark) using 2.5 mg IgG per gram of agarose according to the manufacturers instructions. Columns were prepared using 1 ml of anti-IgG4 antibody-agarose beads. For preparative purposes, 200 μl of 20% (v/v) IgE-depleted serum in assay buffer (PBS pH 7.4 containing 0.025% gelatin (type A from porcine skin (Sigma, St. Louis, MO, USA)) and 0.1% Triton X-100 (Sigma)) was applied to the column, followed by 450 μl of assay buffer. After 1 h, non-bound material was eluted with 2 ml of assay buffer and the column was subsequently washed with 4 ml of 0.9% NaCl. Bound material was eluted in fractions of 0.5 ml using 0.1 M glycine-HCl, 0.15% bovine serum albumin (BSA, Sigma), pH 2.5, followed by neutralisation with 0.075 ml of 1 M Tris, pH 8.2 (Sigma). Acid eluted IgG4 was recovered in a final volume of 3.45 ml. The specificity of the anti-IgG4 affinity column with respect to IgG4 was confirmed in ELISA for IgG1 and IgG4 using 1000 times diluted sheep anti-human IgG1 and IgG4 (The Binding Site, Birmingham, UK) as catching antibodies and horse radish-peroxidase coupled sheep anti-human IgG1 and IgG4 (The Binding Site) diluted 1000 and 500 times, respectively, as secondary antibody.

2.3.3. Depletion of IgG

To prepare serum for determination of the non-IgE, non-IgG-binding of Bet v 1, IgE-depleted serum was fractionated in the following way. Two hundred microliters of the material was run on a column containing 0.5 ml of protein G Sepharose CL-4B (Pharmacia) followed by $2 \times 100 \mu\text{l}$ of assay buffer. Non-absorbed material was eluted in 1.6 ml of assay buffer. Recovery was more than 95% detected as previously described (Svenson et al., 1998).

2.4. Assays for Bet v 1-binding to serum, IgE and IgG

2.4.1. Assay procedure

All dilutions were made in assay buffer and the binding tests were carried out in duplicates. Before detection, samples were pre-incubated for 18 h with ^{125}I -rBet v 1 with or without additions of unlabelled rBet v 1. All analyses were made at 4 °C and the results were corrected for unspecific-binding detected in the presence of 200–500 ng/ml of rBet v 1. Radioactivity (cpm) was determined

with errors below 2% in a gamma counter (Wizard 1470 automatic gamma counter, Wallac, OY, Finland). Measurements of the binding strength (dissociation constant K_d) and binding capacity (B_{max}) were performed by saturation-binding analysis, as described (Svenson et al., 1993). Calculations were performed using GraphPad Prism ver. 4 for curve-fitting (one-site binding, hyperbola).

2.4.2. Binding to serum

Total bound and free ^{125}I -Bet v 1 in 100–300 μl samples were measured by molecular size separation on Sephadex G-75 superfine columns (Pharmacia).

2.4.3. IgG-mediated inhibition of rBet v 1-binding to IgE

IgG-mediated inhibition of ^{125}I -rBet v 1 binding to IgE, was detected using autologous serum IgE bound to anti-IgE coupled paramagnetic beads (ALK-Abelló). A constant amount of bound IgE (binding 30–50% of the added ^{125}I -rBet v 1) was added to individual tubes and washed two times with 1 ml of assay buffer. After careful removal of residual assay buffer, 100 μl of serial dilutions of fractionated IgG were added followed by 20 μl of ^{125}I -rBet v 1 (5000 cpm). After 18 h of incubation, the beads were washed twice and the bound ^{125}I -rBet v 1 detected. Unspecific binding was always less than 1% of cpm added. Inter- and intra-assay variation was below 15%.

2.4.4. Binding to fractionated IgG

Binding of ^{125}I -rBet v 1 to IgG was determined by affinity separation applying 150 μl of serial dilutions of fractionated IgG with rBet v 1 added on 0.5 ml protein G Sepharose CL-4B (Pharmacia). Non-bound ^{125}I -rBet v 1 was washed away with 3 ml of assay buffer, and bound ^{125}I -rBet v 1 was subsequently eluted with 3 ml of 0.1 M glycine-HCl, 0.15% bovine serum albumin (Sigma), pH 2.5. Cpm in both fractions were determined for estimation of bound and free rBet v 1. The background binding was below 2% of cpm added. Inter- and intra-assay variation was below 15%.

2.4.5. Calculations of relative binding and relative blocking activities

Binding curves for unseparated IgG (IgE-depleted serum), IgG4-depleted IgG and purified IgG4 were prepared correcting for the dilution factors resulting from the separation procedure (Fig. 1). The relative binding activities of the two IgG fractions were calculated as the displacement of binding curves compared to unseparated IgG. The arithmetic mean of three determinations at different dilutions in the linear area of the curves was calculated. Similar calculations were performed for the inhibition of IgE binding.

2.5. Statistics

The Wilcoxon matched pairs test was used. *P*-values of less than 0.05 were considered significant.

3. Results

3.1. Binding of rBet v 1 to serum, IgG and IgE

All patients showed more than five-fold increases in IgG binding to rBet v 1 following SIT, whereas specific IgE increased less than two times (data not shown), indicating that all patients responded to SIT.

To test for the serum factors responsible for rBet v 1 binding, the effect of IgE- and IgG-depletion of sera from SIT-treated patients was measured. Binding activity of serum was assayed by molecular size chromatography. When serum was depleted of both IgE and IgG, less than 2% of the serum binding activity remained ($n = 5$). Furthermore, when comparing the binding activities of IgG and IgE more than 50-fold higher activities of IgG were found ($n = 5$). Thus, at least 98% of rBet v 1 binding to sera of SIT-treated patients was associated with IgG and IgE, with IgG as the dominant binding factor.

3.2. Fractionation of sera

Based on the results above, further analyses of allergen-specific IgG in patient sera were made using three different serum fractions: sera depleted of IgE, sera depleted of IgE and IgG4, and purified IgG4. The separation of IgE-depleted serum into IgG4-depleted IgG and purified IgG4 was successful. No IgG1 was found in the IgG4 fraction and IgG4 was depleted to below 7% in all samples (mean 2.3%, data not shown). More than 90% IgG4 was recovered in the purified fraction.

3.3. Binding and blocking activities in IgG-fractions

To measure rBet v 1 binding activities in the three fractions from individual sera, each fraction was tested in serial dilutions as illustrated in Fig. 1A. Furthermore, the blocking activity of the three serum fractions was determined as inhibition of ^{125}I -rBet v 1 binding to autologous IgE (Fig. 1B). The relative contributions of IgG4-depleted IgG and purified IgG4, to the total IgG activity in the two assays, were calculated as the parallel displacement of binding and inhibition curves from the two fractions relative to unseparated IgG. When different separations of the same serum were tested the relative binding and blocking activities were consistent with a variation below 15% (data not shown, $n = 3$). Table 1 shows data from five patients treated with SIT for 30 weeks and nine patients treated for 5 years. In three patients, the relative blocking activity could not be calculated in all IgG fractions. Blocking activity was present, but too low to produce the blocking curves required for quantification using these methods.

As seen in Table 1, from 0.09 to 0.79 of the relative binding activity and from 0.11 to 0.87 of the relative blocking activity could be recovered in the fractions containing IgG4-depleted IgG. From the data in Table 1, it can be

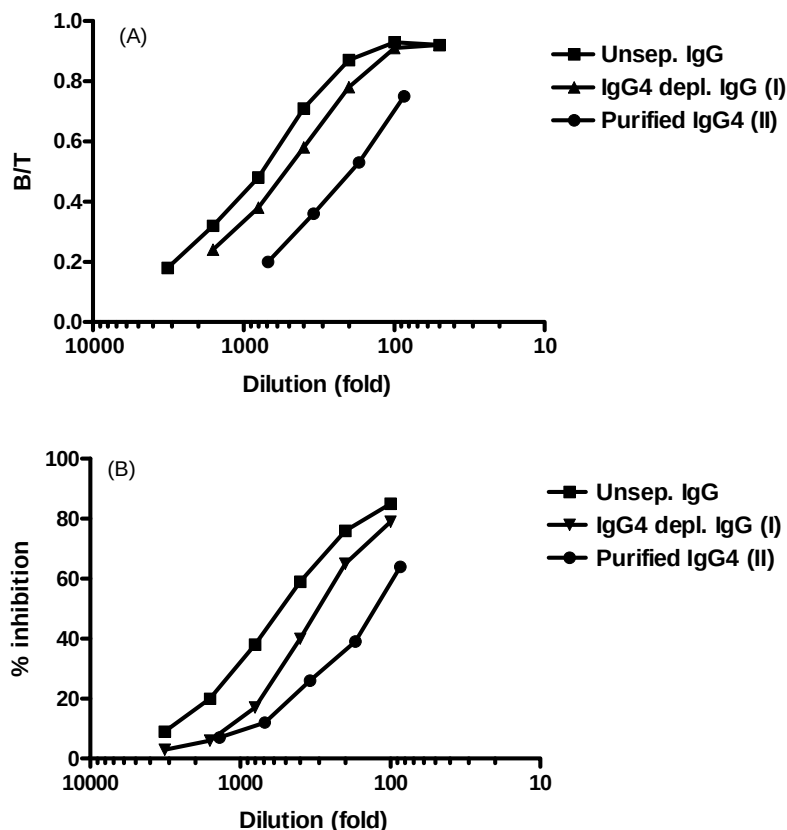


Fig. 1. (A) Representative example of rBet v 1 binding to unseparated IgG, IgG4-depleted IgG (fraction I) and purified IgG4 (fraction II) from a SIT-treated birch pollen allergic patient (#3). IgE-depleted serum was fractionated on an anti-IgG4 column. Serial diluted fractions of IgG were incubated with ^{125}I -rBet v 1. Bound and free ^{125}I -rBet v 1 were separated by absorption to Protein G. Data are shown as mean values of duplicates. Dilution factors of IgG fractions are relative to unseparated serum, based on dilution factors in the separation. B/T: cpm bound/cpm total. (B) Effect on rBet v 1 binding to autologous IgE of unseparated IgG, IgG4-depleted IgG and purified IgG4 from the same patient as shown in (A). Autologous IgE was absorbed to a-IgE paramagnetic beads and washed. Incubations were made with IgG fractions and rBet v 1. Data are shown as mean values of duplicate experiments. Dilution factors of IgG fractions are relative to unseparated serum based on dilution factors in the separation. Percent inhibition is calculated as $((\text{cpm bound in the absence of IgG} - \text{cpm bound in the presence of IgG}) / \text{cpm bound in the absence of IgG}) \times 100\%$.

calculated that the sums of the recovered relative activities in the IgG4-depleted IgG and the purified IgG4 fractions were similar in the two assays, ranging from 0.74 to 0.96 and from 0.75 to 1.32 for the relative binding and blocking activities, respectively.

3.4. Relationship between the relative blocking activity and the relative binding activity

Next, we examined whether there is a relationship between the relative allergen binding and the relative blocking activity, respectively, of both IgG4-depleted IgG as well as purified IgG4. In the IgG4-depleted IgG fractions the ratios between the relative blocking and binding activity varied between 0.81 and 1.4 (Table 1). No statistical difference was found between the relative activities in the two assays (Wilcoxon paired test, $n = 12$, $P = 0.32$) indicating that depletion of IgG4 results in equal reductions in blocking and binding activities. In the case of the purified IgG4 fractions, the median ratio between the activities in the two assays was

1.4 (Table 1) and the relative blocking activity significantly exceeded the relative binding activity (Wilcoxon paired test $n = 13$, $P = 0.001$), suggesting a difference between investigating the contribution of IgG4 to the blocking and binding activities directly by analyzing the purified IgG4 fractions and indirectly as above. The difference in the median blocking/binding ratios between the IgG4-depleted IgG and the purified IgG4 was less than two-fold but statistically significant (Table 1).

3.5. Binding analysis of IgG4-depleted IgG and purified IgG4

Since the determination of binding activities does not discriminate between differences in binding capacities or binding avidities ($1/K_d$) of the samples, we decided to calculate the K_d values of IgG4-depleted IgG and purified IgG4 from saturation binding analyses. Serum samples from seven patients were randomly selected for this analysis. Less than six-fold differences were found between individual patient

Table 1
Binding and blocking activities of IgG4-depleted IgG (I) and purified IgG4 (II)

Patient	IgG fraction ^a	Binding	Blocking	Blocking/binding	K_d (ng/ml) ^b	K_d I/ K_d II
1	I	0.10	0.11	1.1	0.14 ± 0.027	0.67
	II	0.64	0.64	1.0	0.21 ± 0.023	
2	I	0.79	0.78	0.99	0.42 ± 0.093	1.6
	II	0.17	0.2	1.2	0.26 ± 0.052	
3	I	0.65	0.54	0.83	0.60 ± 0.062	1.0
	II	0.26	0.3	1.2	0.58 ± 0.076	
4	I	0.46	0.46	1.0	0.31 ± 0.053	1.3
	II	0.50	0.65	1.3	0.24 ± 0.035	
5	I	0.21	0.17	0.81	ND	
	II	0.68	0.66	0.97	ND	
6	I	0.15	0.19	1.3	0.32 ± 0.048	0.80
	II	0.70	0.72	1.0	0.40 ± 0.052	
7	I	0.24	0.21	0.88	ND	
	II	0.53	0.73	1.4	ND	
8	I	0.32	0.35	1.1	ND	
	II	0.46	0.74	1.6	ND	
9	I	0.61	0.86	1.4	ND	
	II	0.25	0.41	1.6	ND	
10	I	0.20	ND	ND	0.24 ± 0.038	1.1
	II	0.62	0.97	1.6	0.22 ± 0.047	
11	I	0.09	ND	ND	ND	
	II	0.77	1.1	1.4	ND	
12	I	0.67	0.87	1.3	ND	
	II	0.09	ND	ND	ND	
13	I	0.34	0.40	1.2	0.57 ± 0.13	0.75
	II	0.55	0.92	1.7	0.76 ± 0.12	
14	I	0.49	0.53	1.1	ND	
	II	0.31	0.77	2.5	ND	
Median	I			1.1		1.0
	II			1.4		
	I/II					
p^*	I vs. II			0.014	0.93	

Relationship between the relative binding activities of rBet v 1 to IgG4-depleted IgG and purified IgG4, and the relative blocking activities of the IgG fractions on rBet v 1 binding to autologous IgE. K_d values measured in the IgG fractions by saturation analysis. Patient 1–5 treated with SIT for 30 weeks. Patient 6–14 treated with SIT for 5 years. ND: not determined.

^a Fraction I: IgG4-depleted IgG, fraction II: purified IgG4.

^b K_d values $\pm$ S.E.M.

* Wilcoxon paired test.

K_d values (0.14–0.76 ng/ml) (Table 1). The ratios between the K_d values of IgG4-depleted IgG and purified IgG4 varied between 0.67 and 1.6 and no statistical difference was found between the K_d values of the two IgG fractions (Table 1).

4. Discussion

The molecular and cellular immunological mechanisms underlying specific immunotherapy are not yet fully understood. Successful SIT is most often accompanied by an increase in allergen-specific antibody. However, attempts to correlate quantitative changes in allergen-specific IgG

and IgG subclasses with clinical outcome of SIT have not reached consensus (Hedlin et al., 1986; Ohashi et al., 1987; Nakagawa et al., 1987; Djurup and Malling, 1987; Malling and Djurup, 1988; Pastorello et al., 1992; Gehlhar et al., 1999). On the other hand, a dominant contribution in the blocking activity of specific IgG has been attributed to IgG4 (Ohashi et al., 1987; Leynadier et al., 1991; Lambin et al., 1993; Panzani et al., 1996). It has therefore been suggested that qualitative changes in IgG and IgG subclasses such as affinity maturation and epitope spreading may take place during SIT, particularly concerning the IgG4 subclass (Denepoux et al., 2000; Larsen et al., 2000). Consequently, we investigated the binding of Bet v 1 to IgG and IgE to

compare the intrinsic blocking activity of unseparated IgG, IgG4-depleted IgG and purified IgG4 from SIT-treated birch pollen-allergic patients. We used rBet v 1.2801, a recombinant variant of the major allergen of *B. verrucosa* pollen. This variant has been shown to be a relevant substitute for natural Bet v 1 in immunochemical assays, since it contains most of the epitopes relevant for the binding of human IgE (Spangfort et al., 1996; Svenson et al., 2003).

In this study, a selective increase in allergen-specific serum IgG and a dominant binding of rBet v 1 to this immunoglobulin class were observed for all the SIT-treated patients, as previously reported (Svenson et al., 2003). When depleted of IgG4, the individual sera showed a 10–90% reduction in binding and blocking activity of the IgG anti-rBet v 1, and when adding the activities of the two IgG fractions, the recoveries were from 75 to 130%. This shows that a substantial part of the IgG anti-rBet v 1 activity following SIT may not be of the IgG4 subclass. Furthermore, we did not find a significant difference between the K_d values of IgG4-depleted IgG in serum and the purified IgG4. The K_d values were in the same range as those measured in other allergen systems and K_d values found in individual IgG clones, and showed small inter-individual variation (Adkinson et al., 1979; Prahl et al., 1981; van der Zee et al., 1986b; Visco et al., 1996; Jackola et al., 2002; Svenson et al., 2003). In ragweed-allergic patients that were not SIT-treated, no differences in the avidity of Amb a 1-specific IgG1 and IgG4 were found (Jackola et al., 2002), and comparing individual sera with specific IgG almost exclusively belonging to either the IgG1 or the IgG4 subclass, no differences in avidity were found (van der Zee et al., 1986b). Because no or marginal changes in Bet v 1 K_d values of polyclonal IgG from individuals have been observed following SIT (Svenson et al., 2003), our results do not suggest that affinity maturation of specific IgG4, or of other IgG subclasses, is of importance in SIT-induced IgG binding of allergen. Rather, our results support the original suggestion by Adkinson et al. (1979), that variable K_d is not a major contributor to the IgG-allergen-binding capacity of most sera. However, detected by assay systems other than those used here, immunoglobulin subclass and isotype-restricted differences in avidities have been reported, depending on the atopic status of the individuals (Devey et al., 1989; Jackola et al., 2002). The highest avidities were reported for allergen-specific IgG1 from non-atopic individuals; and in atopic individuals, specific IgE appeared to display nearly 10 times higher avidities than specific IgG and IgA (Jackola et al., 2002). In SIT-treated bee venom atopics the avidity of IgG4 was higher than that of IgG1, and IgE anti-phospholipase A2 antibodies were reduced in avidity following SIT (Devey et al., 1989). Comparing findings in insect venom and pollen allergic individuals, the different routes of sensitization should however be considered.

Heterogeneity in epitope recognition by IgE of individual patients has been shown (Spangfort et al., 2003), an aspect that may complicate predictions of the influence of differ-

ent treatments on IgE binding of allergens. Monoclonal human Bet v 1-specific IgG obtained from an allergic patient after successful SIT have shown that different clones may exert blocking, non-blocking or even enhancing effects on IgE allergen binding (Visco et al., 1996; Denepoux et al., 2000). Interestingly, it has been found that mixing clones of non-blocking IgG may result in blockade of Bet v 1 binding to IgE (Visco et al., 1996).

Also differences between individuals in cross-binding homologous structures of Bet v 1, such as the apple allergen Mal d 1, is observed with both SIT and pre-SIT serum IgG from birch pollen-allergic patients, but nevertheless no difference in the IgG blocking activity of Bet v 1 binding to the patients IgE was observed (Svenson et al., 2003). These observations suggest that differences in epitope binding of individual clones have minor importance when looking at the blocking activity of allergen-specific polyclonal IgG.

Our results show that IgG4 depletion resulted in equivalent reductions in binding and blocking activities. In contrast, a significant but less than two-fold higher relative blocking than binding activity was found in the purified IgG4 fraction. Unseparated IgG was used as reference, and in most cases IgG4 appeared to be the dominant contributor of both the blocking and binding activity. On this background, one would rather have expected a result showing equal relative blocking and binding activities of purified IgG4. This discrepancy might indicate that the purification may have changed the characteristics of IgG4, or that serum contained factors interfering with the blocking activity of IgG4. However, the ratio between relative blocking and binding activities of purified IgG4 differed less than two-fold from those of IgG4-depleted IgG, and as mentioned above, IgG4 depletion had no effect on the ratio between relative blocking and binding activities of IgG. Therefore, in the case of Bet v 1-specific IgG from SIT-treated patients, and possibly also for other allergens, we find it most likely that similar relative blocking activities are found in different subclasses of specific IgG. This view is not contradicted by observations of IgG4 as a dominant blocking factor of serum from SIT-treated patients (Leynadier et al., 1991; Lambin et al., 1993). In filariasis for example, histamine release was blocked by both specific IgG1 and IgG4 from the patients (Hussain et al., 1992), and blocking of IgE-facilitated antigen presentation has been found not to correlate with the content of allergen-specific IgG4 in sera (van Neerven et al., 1999).

Although qualitatively, the blocking activities of specific IgG4 and non-IgG4 seem to be of similar magnitude, differences in the effector functions of IgG subclasses may be of importance for the clinical effect of SIT. IgG4 has been shown to be functionally monovalent, which results in the formation of small non-precipitating immune complexes with low potential for inducing immune inflammation (van der Zee et al., 1986b; Schuurman et al., 1999; Aalberse and Schuurman, 2002). Furthermore, the binding of IgG4 to Fcγ receptors is generally weak compared to other

IgG subclasses (reviewed in Ravetch and Kinet, 1991; Hulett and Hogarth, 1994). Another feature of IgG4 is the inability to fix complement, and IgG4 indeed has been shown to inhibit complement activation by IgG1 antibodies (van der Zee et al., 1986a); this mechanism may protect against complement-induced damage.

In conclusion, we find that SIT-induced Bet v 1-specific IgG4 contributes to IgG blocking of allergen–IgE interaction in a simple quantitative manner and not by a particular intrinsic blocking activity.

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