

A double-blind, placebo-controlled birch allergy vaccination study: inhibition of CD23-mediated serum-immunoglobulin E-facilitated allergen presentation

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Summary

Background The clinical efficacy of specific allergy vaccination (SAV), previously called specific immunotherapy is well documented. The working mechanism of this treatment is not completely known at present. Allergen-specific CD4⁺ T lymphocytes are activated at extremely low allergen concentrations *in vivo* possibly as a result of serum IgE-facilitated allergen presentation (S-FAP). Previously, we have shown that this process can be inhibited by long-term birch SAV sera.

Methods In the present study, we have analysed sera from birch-allergic patients in a randomized double-blind, placebo-controlled clinical trial for their ability to mediate S-FAP. Birch-specific IgE levels were not changed after SAV. Bet v 1-specific IgE levels, however, were significantly decreased ($P < 0.05$) and Bet v 1-specific IgG4 levels were strongly increased after SAV ($P < 0.001$). None of these changes were observed in the placebo group. When the sera were tested for their ability to induce S-FAP, a complete abrogation of this effect was noted in the sera from patients receiving active treatment ($P < 0.001$), but not in the control group. This inhibition of S-FAP seemed to be associated with the reduction in the ratio between Bet v 1-specific IgE and IgG4 antibodies in serum, but a clear correlation could not be demonstrated.

Conclusion In conclusion, the present study clearly shows that SAV leads to an inhibition of the S-FAP needed to obtain optimal T cell activation at the low allergen concentrations present *in vivo*. This novel mechanism may explain the increased allergen threshold levels found in allergen provocation tests and the reduction of late-phase reactions observed after SAV.

Keywords allergy vaccination, Bet v 1, CD23, serum-facilitated allergen presentation, T lymphocyte. Submitted 6 May 2003; revised 10 October 2003; accepted 17 November 2003

Introduction

Specific allergy vaccination (SAV) [1], has been practised for almost a century [2], and is currently the only allergy treatment that reduces symptoms through a modulation of the course of the allergic disease itself [1]. The clinical efficacy of SAV is well documented, and clear effects are noted with respect to symptom/medication scores, early- and late-phase skin responses, and increased allergen threshold levels in provocation tests. Moreover, SAV has been shown to have long-term efficacy [3, 4] even several years after withdrawal [5], reduce the risk of developing new sensitizations [6], and have been suggested to inhibit the progression from rhinitis to asthma [7].

Even though the precise immunological mechanisms underlying SAV are a matter of debate, several immunological effects have been described. In general, specific serum-IgE

levels do not decrease after SAV but rather show a transient increase [8] and a significant increase in allergen-specific IgG antibodies [9, 10] including IgG4 [11–13] has been reported. Changes in cellular reactivity include a decreased influx of activated eosinophils and activated CD4⁺ T cells into target tissues after topic allergen provocation as well as during the pollen season [14, 15]. A shift in the production of cytokines from Th2- to Th0-type [16–19], a decreased proliferative response [17, 20], and an increased production of IL-10 [21] have been demonstrated for allergen-specific T cells. Furthermore, an increased production of IL-12 [22] and IL-10 [21] by antigen-presenting cells (APCs) has been reported.

However, these immunological changes that SAV induces cannot completely explain the clinical changes described after SAV suggesting that the mechanisms behind SAV may involve changes that connect different components of the immune response, i.e. antibodies, APCs and T cells as observed with serum IgE-facilitated allergen presentation (S-FAP). The fact that the actual exposure to airborne allergens is extremely low, i.e. in the range of a few micrograms per year suggests that a specific antigen uptake is involved in the presentation of allergens to T cells. The IgE Fc receptors CD23 and FcεRI

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are found on APCs and allergic patients have been shown to have an increased expression of CD23 on B cells and macrophages [23, 24], and FcεRI on monocytes and dendritic cells [25, 26].

Indeed, both IgE receptors have been shown to facilitate the presentation of allergens, resulting in T cell activation at 100–1000-fold lower allergen concentrations [27–33], which clearly demonstrates the potential of this cooperation between the humoral and the cellular allergen-specific response in allergic patients.

This study describes the influence of SAV on CD23-mediated serum-IgE-facilitated allergen presentation of Bet v 1 in a placebo-controlled birch SAV study.

Materials and methods

Patients and treatment

The study was designed as a randomized double-blind, placebo-controlled study of SAV in patients allergic to birch pollen and was performed in Gothenburg, Sweden, from autumn 1996 to summer 1998. The clinical results of the study are reported in detail elsewhere [34] and summarized in Table 1. In brief, 49 patients with a history of rhinoconjunctivitis with or without mild-to-moderate asthma to birch pollen were recruited for the study. Twenty-one of the patients were sensitized towards grass pollen in addition to birch. The patients were randomized to treatment with either birch pollen extract (*Betula verrucosa*, Alutard SQ[®], ALK-Abelló A/S, Denmark) or placebo. The maintenance dose (100 000 SQ-U) was reached in 7 weeks using a clustered updosing schedule of weekly injections with 3 or 2 doses each separated by 30 min [34]. The patients were treated for 18 months in the period ranging from December or January 1996/1997 to July 1998 covering the birch pollen seasons of 1997 and 1998.

Sera and Bet v 1-specific IgE and IgG4 measurements

Sera from the actively and placebo treated birch-allergic patients undergoing SAV were obtained before start of treatment and by the end of the study. All sera were blinded, and clinical data were not available by the time of the analyses. Sera from 21 actively treated and 21 placebo-treated patients were available in sufficient amounts for the study.

Allergen-specific IgE and IgG4 was measured using the Magic Lite SQ IgE system (ALK-Abelló A/S, Hørsholm, Denmark) [35].

Preparation of Bet v 1

Bet v 1 was enriched from an aqueous birch pollen extract as described by Ipsen and Lowenstein [36]. In brief, size exclusion chromatography of 150 mg (dry weight) of pollen extract was performed on a G-75 superfine (Pharmacia, Uppsala, Sweden) 90 cm × 2 cm² (K15/100 Pharmacia, Uppsala, Sweden) column, equilibrated with 0.125 NH₄HCO₃, pH 8.3. The fractions containing Bet v 1 (assayed by fused rocket immunoelectrophoresis and fused rocket radio immunoelectrophoresis) were pooled and lyophilized. Typically 15–30 mg of dry weight material was obtained.

Table 1. Summary of clinical results

	Active	Placebo	P-value
Symptoms 1997 (score/pt/day)	1.4	2.2	0.055
Medication 1997 (score/pt/day)	0.7	2.5	0.004
Severity of disease (VAS) 1997 (mm/pt)	11.3	27.7	0.001
Symptoms 1998 (score/pt/day)	2.7	4.5	0.005
Medication 1998 (score/pt/day)	1.4	7.5	0.004
Severity of disease (VAS) 1998 (mm/pt)	21.6	14.9	0.041
Late phase skin response (Δ mm ² /pt)	– 1442.6	– 412.0	0.025
Conjunctival provocation test (CPT) (Δ SQ-U/pt)	0	0	ns
Bronchial allergen provocation test (Δ SQ-U/pt)	761.0	– 116.0	0.003

Seasonal symptoms and mediation scores are calculated on basis of area under the curve analyses. Seasonal severity of disease is analysed from weekly subjective evaluation during the birch pollen seasons in 1997 and 1998. CPT and BAPT are analysed as changes from baseline to one year of treatment. Median values are presented for each parameter in the active and the placebo groups.
ns, non-significant.

Culture conditions

T cells were cultured in RPMI 1640 medium (Gibco, Paisly, UK) supplemented with 2 mM L-glutamine (Gibco), 100 IU/mL of penicillin–streptomycin (Gibco) and 5% heat-inactivated, screened human AB⁺ serum (Bio-Whittaker, Wokingham, Berks, UK). All cultures were grown under sterile conditions at 37 °C in a humidified atmosphere of 5% CO₂. Epstein–Barr virus (EBV)-transformed B cell lines were cultured in RPMI, 2 mM L-glutamine, 100 IU penicillin–streptomycin, and 10% heat-inactivated fetal calf serum (Gibco) under the same culture conditions as for the T cells.

Bet v 1-reactive T cells

Bet v 1-specific T cell line and clones were generated from the peripheral blood of patient AF, who is clinically allergic to birch pollen as well as SPT positive and Magic Lite class II to birch [37]. The CD4⁺ TCC AF28 reacted with aa 21–33, CD4⁺ TCC AF24 with aa 37–51 and AF line (which contains both CD4⁺ and CD8⁺ T cells) reacted with several T cell epitopes simultaneously.

Facilitated antigen presentation

Serum samples from birch-allergic patients were incubated for 1 h at 37 °C with serial dilutions of Bet v 1. Then EBV-B cells (irradiated 5000 rad) were added at a concentration of 1–3 × 10⁶/mL to the allergen–serum mixtures containing Bet v 1-Ig complexes and incubated for 1 h at 37 °C. After incubation, the cells were washed twice in RPMI, and used as APCs in the T cell stimulation assays. For these experiments, Bet v 1-specific T cells were washed twice in RPMI 1640 medium. Antigen-specific proliferation was assessed by stimulation of the T cells, 2–4 × 10⁴ cells/well, using the allergen/serum/B cell preparations described above at 1–2 × 10⁴ B cells/well. The cells were cultured for 24 h, followed by a 16-h pulse in the presence of tritiated methyl thymidine (³H-TdR) (NEN DuPont, Wilmington, DE, USA), 0.5 μCi/well. Proliferation was expressed as mean c.p.m. of ³H-TdR incor-

poration of triplicate cultures. All experiments shown were performed at least two times with AF line and at least one CD4⁺ Bet v 1-specific TCC. The T cell responses were scored at 0.3 µg/ml Bet v 1.

Statistic evaluation of the data

The statistical evaluation of the clinical data were analysed using the Wilcoxon two-sample rank-sum test. Estimation of mean differences and 95% confidence limits for this have been presented using the Hodges–Lehmann estimation. The statistical analyses of specific cellular and antibody responses were performed by paired *t*-tests. *P*-values below 5% were considered significant.

Approvals

Approvals from the Swedish Medical Products Agency and the Ethics Committee and signed informed consent were obtained prior to starting the trial. The trial was conducted in accordance with the Helsinki Declaration and with good clinical practice (GCP).

Results

Summary of clinical results

The clinical results from the study are described in detail elsewhere [34] and summarized in Table 1. The primary endpoint for evaluation of clinical efficacy is seasonal symptoms and medication use. The results demonstrate that after only a few months of treatment there was a significant reduction in medication (72.1%) and a tendency in reduction of symptoms (38.2%) in the active group as compared with the placebo group even though the pollen season of 1997 was very poor with a peak of only 450 pollen/m³. Results from dairy card obtained in 1998 shows significant efficacy of a 40% ($P <$

0.005) reduction in symptom score and 81.4% ($P < 0.005$) reduction in drug consumption (peak pollen more than 5000 pollen/m³). There was a highly significant difference ($P < 0.001$) between groups in subjective scoring of the severity of disease during the pollen season of 1997 as evaluated by a visual analogue scale (VAS). In 1998, it is seen that both groups felt worse but the active group still scored significantly lower than the placebo ($P < 0.05$). The late cutaneous response to intradermal injection of 50 SQ-U birch pollen extracts was significantly smaller in the active group as compared with the placebo ($P < 0.03$). Furthermore, specific allergen bronchial provocation test performed in the subgroup of the asthmatic patients (separately randomized) also documented an increased bronchial tolerance to inhaled birch pollen extract ($P < 0.001$). In spite of significant efficacy of SAV on hayfever symptoms there was no detectable difference in specific conjunctival provocation test with allergen.

Allergen-specific IgE

To investigate the effect of SAV on specific IgE levels, the serum concentrations of IgE specific to the major allergen Bet v 1 was analysed before and after treatment (Fig. 1). There were no significant changes in specific IgE to Bet v 1, between the active and the placebo group from before to after treatment as analysed. However, there was a significant reduction of the Bet v 1-specific IgE levels in the actively treated group comparing sera from before with sera drawn after treatment ($P < 0.05$). As a control, the sera were analysed for specific IgE to the major allergen from Timothy pollen, Phl p 5. There was no change between groups or from baseline to the end of study in either group (data not shown).

Allergen-specific IgG4

The analysis of specific IgG4 to Bet v 1 is shown in Fig. 2. It is evident that there is a highly significant increase in serum

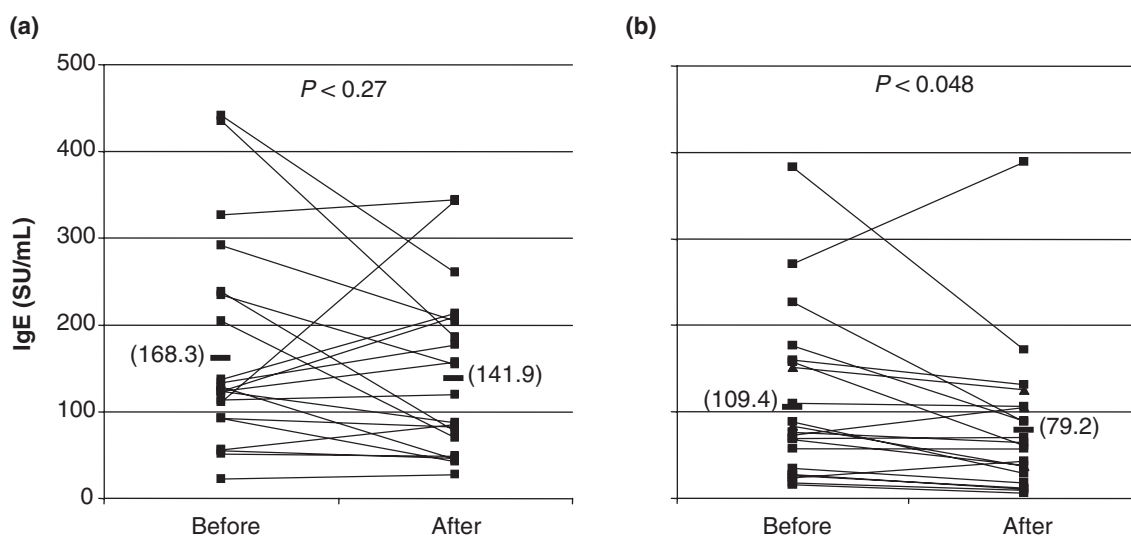


Fig. 1. Allergen-specific serum IgE levels are reduced after SAV. Serum concentrations of Bet v 1-specific IgE in actively (b) and placebo-treated patients (a) were measured out of season before and after 18–24 months of SAV using the MagicLite system. The three patients with low inhibition of S-FAP after active treatment (see Fig. 3b) are indicated by triangles. *P*-values from a paired *t*-test for means are indicated. No significant differences were observed between groups at the two time-points.

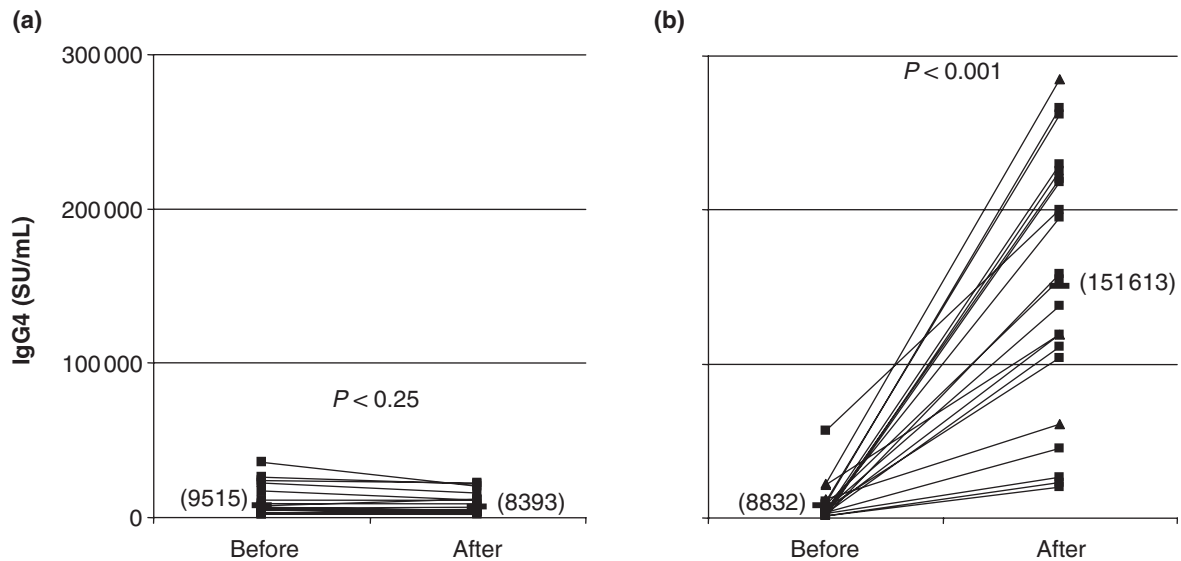


Fig. 2. Allergen-Specific Serum IgG4 levels increase as a result of SAV. Serum concentrations of Bet v 1-specific IgG4 actively (b) and placebo-treated patients (a) were measured out of season before and after 18–24 months of SAV using the MagicLite system. The three patients with low inhibition of S-FAP after active treatment (see Fig. 3b) are indicated by triangles, *P*-values from a paired *t*-test for means are indicated. No significant differences were observed between groups before treatment, while the mean values differed significantly after treatment ($P < 0.001$).

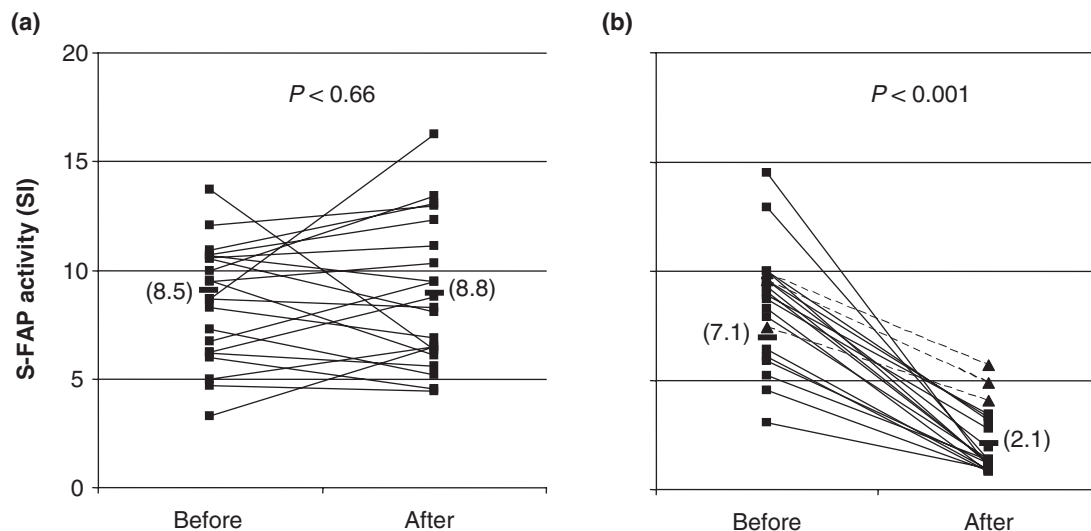


Fig. 3. Serum facilitated allergen presentation is strongly reduced after SAV. Bet v 1 S-FAP in actively (b) and placebo-treated patients (a) were measured in serum samples drawn out of season before and after 18–24 months SAV. The experiments were reproduced in comparable set-ups using a Bet v 1-specific T cell line and clones. The three patients with low inhibition of S-FAP after active treatment are indicated by triangles. *P*-values from a paired *t*-test for means are indicated. No significant differences were observed between groups before treatment, while the mean values differed significantly after treatment ($P < 0.001$).

IgG4 in the actively treated group ($P < 0.001$) while no change was observed in the placebo-treated patients ($P < 0.25$) from baseline to the end of study. However, based on the reactivity of the individual patients it can be seen that patients 11, 12, and 16 in the active group exhibit considerably higher before-treatment levels than the rest of that group. In spite of their high pre-treatment levels they show strong increases in Bet v 1-specific IgG4 as a result of the treatment comparable with the rest of the actively treated patients (Fig. 2b). There was no change in Phl p5 sIgG4 in the two groups from baseline to end of treatment. The fact that only the level of Bet v

1-specific antibodies changed as a result of SAV clearly document the specificity of the treatment (data not shown).

S-FAP

To evaluate the influence of specific allergen vaccination on S-FAP, sera from the study patients were tested for their ability to facilitate the presentation of Bet v 1 to Bet v 1-reactive T cells. As previously demonstrated, only sera containing Bet v 1-specific IgE antibodies influenced the T cell activation, while the presence of control sera containing grass allergen-specific

IgE antibodies drawn before or after grass-SAV had no effect on activation of the Bet v 1-specific T cells [33]. The analyses were performed on sera obtained before start of treatment and at the end of study (Fig. 3). It is clear that SAV induces a highly significant inhibition of S-FAP. In the actively treated group sera from all patients, except three, exhibit more than 50% inhibition of S-FAP compared with pre-treatment S-FAP activity. These are patients 12, 16, and 36 and they were also the only ones still inducing relatively strong S-FAP-mediated T cell activation with SI-values above 4.

Correlation between Bet v 1-specific serum antibody concentrations and S-FAP

Both the Bet v 1-specific IgE and IgG4 levels are important for the T cell activation obtained and the most straightforward explanation for the reduction of S-FAP observed is a competition between these two allergen-specific Ig-isotypes for allergen binding. Therefore, we investigated the relationship between the IgE/IgG4 ratio and the T cell activation obtained in the two groups before and after treatment (Figs 4a and b). It is obvious that the two groups have similar IgE/IgG4 ratios in serum and that comparable T cell activation is induced by the sera obtained before treatment (Fig.

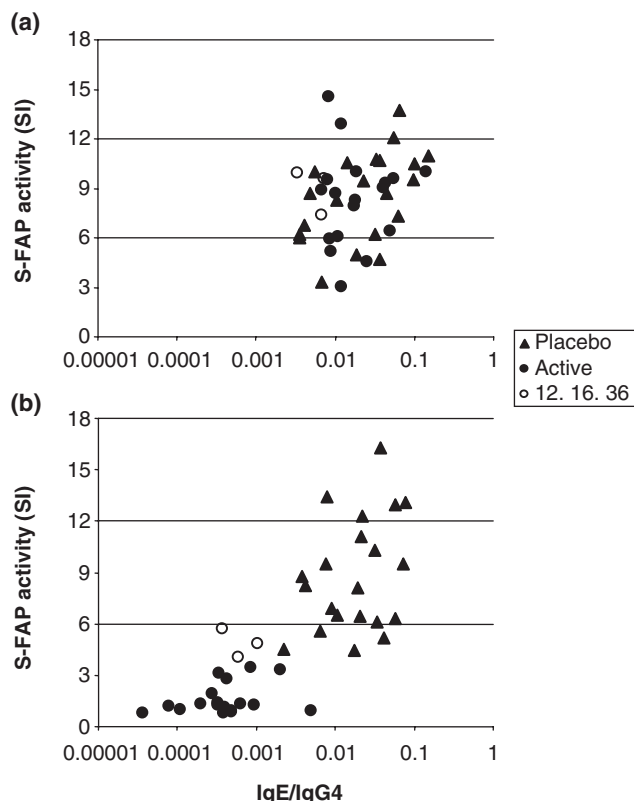


Fig. 4. Serum facilitated allergen presentation and Bet v 1-specific antibody levels for individual patients do not correlate. The relationship between Bet v 1 S-FAP and serum IgE/IgG4 ratios was assessed. Data obtained from serum samples from placebo (triangles) and actively (squares) treated patients drawn before (a) and after (b) SAV were investigated. Patients 12, 16, and 36 from the actively treated group showed only a marginal reduction in S-FAP (see Fig. 3b) and are indicated separately by open circles. No significant correlations were observed in the different groups before or after SAV or in the total patient material.

4a). In addition, it seems that sera from most patients induce strong S-FAP mediated T cell activation resulting in a horizontal distribution of the observations in Fig. 4a. This could indicate that all sera contain saturating IgE-concentrations able to efficiently compete with IgG4 for binding to Bet v 1 and the subsequent formation of allergen-Ig complexes. After treatment, the IgE/IgG4 ratio is unchanged in the placebo group, while sera from patients receiving active treatment show a 10- to 100-fold reduction in this ratio (Fig. 4b). This prominent change in Bet v 1-specific serum Ig isotypes was coincident with the strong reduction in S-FAP, but still a direct correlation on the single-patient level was not found. However, the T cell activation obtained seemed to reflect the IgE/IgG4 ratio more closely in the actively treated group after treatment (Fig. 4b). Similarly, no direct correlation was found when the relation between T cell activation and the Bet v 1-specific IgE or IgG4 was investigated individually.

Correlation of S-FAP and clinical efficacy

The clinical efficacy of the SAV treatment was evaluated by changes in symptom and medication score and showed a

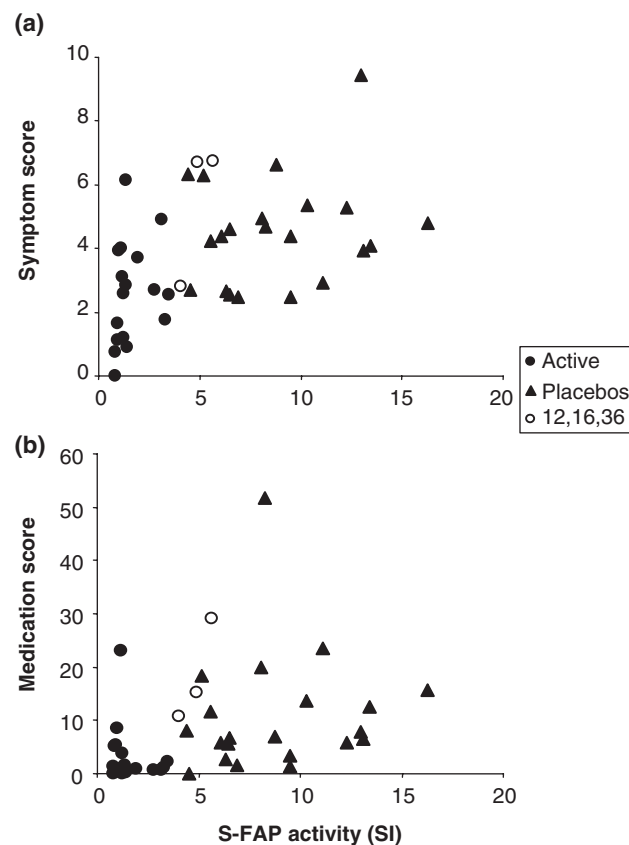


Fig. 5. Symptom and medication scores (score/pt/day) for individual patients are not directly correlated to S-FAP activity in serum. The relationship between Bet v 1 S-FAP and symptom (a) and medication (b) scores was assessed. Data obtained from serum samples from placebo (triangles) and actively (squares) treated patients drawn before and after SAV were investigated. Patients 12, 16, and 36 from the actively treated group showed only a marginal reduction in S-FAP (see Fig. 3b) and are indicated separately by open circles. No significant correlations were observed in the different groups before or after SAV or in the total patient material.

highly significant improvement of the patient's symptoms and reduction of drug consumption as a result of treatment (Table 1) [34]. It would be of great value to obtain a prediction parameter for the success of treatment. We investigated the relationship between S-FAP-mediated T cell activation and medication or symptom score. Even though there was a clearly significant reduction of symptoms in the actively treated group it was not possible, on the single-patient basis, to show a direct linkage to the level of T cell activation obtained via serum antibodies (Fig. 5a). Within the group of actively treated patients relatively high symptom scores are obtained by patients with completely inhibited S-FAP and in the placebo group low symptom scores are found in patients with high S-FAP activity in serum. Two of the three actively treated patients (patients 12 and 16) that still had serum components that facilitated significant T cell activation had the highest symptom score but were not clearly separated from the rest of the treated patients while the third patient (patient 36) reported low-to-medium symptoms.

Late-phase reactions may be the main reason for drug consumption in relation to allergic disease and activation of allergen-specific T cells plays an important role in this part of the allergic response. This could suggest that the S-FAP-mediated T cell activation and the medication score would be closely related.

Only four patients had a medication score above 10 and all three patients with serum inducing significant S-FAP after active treatment were found among these four patients (Fig. 5b). This could suggest that a high potential for S-FAP leads to high medication intake. However, 13 of the placebo-treated patients with sera inducing high S-FAP mediated T cell activation reported moderate medication intake resulting in medication scores below 10, which argues against a linkage between S-FAP and medication use.

Discussion

IgE-mediated activation of allergen-specific T cells through allergen focussing on CD23⁺ or FcεRI⁺ APC has been suggested to be the most likely mechanism of T cell activation at the low allergen doses to which allergic patients are exposed. This mechanism, known as S-FAP, was clearly inhibited by allergen-specific IgG fraction of sera from patients that had received SAV [33].

In the present study, we have confirmed these findings in a large group of birch-allergic patients enrolled in a placebo-controlled, double-blind SAV study. Arvidsson et al. previously reported a highly significant clinical effect in the actively treated patients [34] (Table 1). Complete abrogation of S-FAP, was only observed in the patient group receiving active treatment (Fig. 3a and b). In addition, we found a highly significant increase in allergen-specific IgG4 in SAV-treated patients associated with the clear inhibition of S-FAP. This indicates that the binding of allergen-specific IgG4 to the allergen in competition with IgE reduces the formation of allergen-IgE complexes needed for S-FAP and causes decreased facilitation of T cell activation by IgE at low allergen concentrations. The induction of IgG4 observed in connection with SAV may therefore be involved in the

reduction of T cell-mediated late-phase allergic symptoms. In addition, these changes may explain the observed increase in the allergen threshold after skin provocation in SAV-treated patients, which also was observed in the present study (Table 1).

In 18 out of 21 patients receiving active treatment, there was a clear connection between medication score and inhibition of S-FAP, thus suggesting that S-FAP may be used to monitor and/or predict the outcome of SAV on the basis of an *in vitro* analysis early in the course of treatment. Only sera from three patients receiving active treatment were not completely inhibited in their ability to mediate strong S-FAP after treatment (SI > 4). Interestingly, these patients seemed to be low responders demonstrated by high symptom and medication score (open circles, Fig. 5a and b). Another patient had a high medication score after treatment even though the induction of T cell activation by this patient's serum was completely inhibited and several patients with complete inhibition of S-FAP after treatment still obtained relatively high symptom scores. This indicates that inhibition of S-FAP correlates with clinical improvement, but that on a single-patient level there is not a 100% concordance between clinical status and the occurrence of S-FAP.

A more direct comparison might be based on the relationship between changes in S-FAP and changes in symptom and medication scores, but baseline data on the clinical parameters were not available in the current investigation.

A connection between IgG4 and successful SAV has previously been demonstrated in several reports even though the rise in specific IgG4 antibodies after SAV does not correlate directly with clinical efficacy [1, 5, 38, 39]. In contrast, other groups report that high IgG4 levels are related to a poor outcome of SAV [12]. The present study may indicate that rather than allergen-specific IgG4 levels *per se*, the ratio between allergen-specific IgE and IgG4, may be a more useful parameter. We were not able to demonstrate a direct correlation between IgE/IgG4 ratios and the clinical parameters or S-FAP suggesting that the total allergen-specific nonIgE should be taken into account instead of only IgG4. S-FAP reflects the function of the mixture of allergen-specific antibody isotypes present in the patients serum and may be a rational alternative for evaluation of treatment success.

The level of Bet v 1-specific IgE is significantly reduced, which may result in a reduction of S-FAP. On the other hand the mean IgE level is still 79.2 SU/mL, which means that S-FAP activity should still be detectable in the majority of the sera from patients in the actively treated group. This strongly suggests that the reduction in Bet v 1-specific IgE is not the major cause of the reduction in S-FAP.

Minor modifications of the S-FAP analysis may be critical for detecting correlations to clinical or paraclinical parameters. As demonstrated previously, it is possible to measure inhibition of S-FAP activity induced by individual post-SAV sera in indirect S-FAP performed with a mixture of the post-SAV serum and a standard serum containing a high concentration of allergen-specific IgE. The direct S-FAP measurements performed here with the patient's own IgE and IgG4 competing for allergen binding may reflect the *in vivo* situation best. In contrast, the relative or indirect S-FAP may be a

more artificial situation, but it circumvents the variability in serum IgE concentrations of the individual patient and may therefore give a measurement that directly reflects changes in allergen-specific non-IgE antibodies, which may be closely related to clinical changes. In addition, the horizontal distribution of the observations in Fig. 4 may indicate that the saturating IgE-concentrations generally present in sera from birch-allergic patients form optimal allergen-Ig complexes even in the presence of relatively high pre-treatment concentrations of allergen-specific IgG4. The 10- to 100-fold reduction in the IgE/IgG4 ratio as a result of active treatment in combination with the strong inhibition of S-FAP leads to a situation where the T cell activation seemed to reflect the IgE/IgG4 ratio more closely (Fig. 4). On the other hand, in this situation the S-FAP activity in most sera is completely inhibited. This suggests that the S-FAP measurements change from one extreme to another, which makes it difficult to demonstrate a clear correlation between S-FAP and antibody measurements or clinical parameters.

We previously found that S-FAP occur with sera that contained >50 SU/mL Bet v1-specific IgE, indicating that the effect is relevant for the majority of patients undergoing SAV and that changes in the IgE/IgG4 ratio were reflected in the T cell activation as early as after 3 months of treatment [33]. This suggests that the clinical effect of SAV treatment may be predicted at an early stage using this assay method. Recently, it was shown that S-FAP can be evaluated through determination of the level of IgE-allergen complex binding to B cells [40, 41]. The use of B cell binding of allergen complexes as the final readout may make the assay applicable for clinically monitoring the effect of SAV in individual patients. If this induction of allergen-specific non-IgE antibodies is crucial for the success of SAV it should be a key point in the evaluation of new treatment strategies. Modified allergens, allergen fusion proteins, or allergen fragments [42] have to be tested in great detail for their capacity to induce allergen-specific non-IgE antibodies and the effect of the antibodies has to be addressed in a biological relevant test system such as S-FAP-mediated T cell activation.

The reduction of S-FAP or of allergen-IgE complex formation induced by immunotherapy may influence the allergic response at different levels *in vivo*, especially since IgE-allergen complexes have been shown to drive Th2 responses. For example, Th2 responses and specific IgG1 and IgE production in mice are enhanced by allergen-IgE complexes [43, 44] or by allergen-aCD23 mAb conjugates [45]. Further, the production of IL-4 and IL-5 and the recruitment of eosinophils to the lungs of mice sensitized with house dust mite can be inhibited by treatment with an anti-CD23 or anti-IgE mAb. Similarly, Th2 responses and IgE production were eliminated in CD23-KO mice [46, 47].

Clinical trials with a neutralizing anti-IgE mAb have shown that anti-IgE mAb inhibits bronchial late-phase responses and reduces the number of eosinophils in the sputum of allergic asthma patients [48–50], also indicating that inhibition of S-FAP may correlate with clinical improvement [40].

The present data demonstrate that S-FAP is a general mechanism for T cell activation. In combination with the findings described above, this strongly suggests that IgE, as well as IgE receptors, play an important role in the *in vivo* activation of CD4⁺ T cells, the regulation of humoral

responses, and in late-phase allergic responses and underscores the importance of changes in the allergen-specific IgE/non-IgE antibody balance.

In conclusion, we have demonstrated that inhibition of S-FAP as a result of SAV is a general finding and that an assay based on S-FAP measurements might be used to determine the clinical outcome of SAV. In addition, inhibition of allergen-IgE complex formation by blocking antibodies measured by S-FAP influences the allergic response at different levels and such measurements should be included in the evaluation of new treatment protocols for specific treatment of allergic diseases.

How these findings will change the interpretation of results from *in vitro* T cell assays and how SAV-induced changes in facilitated allergen presentation will influence the Th1/Th2 balance of an allergen-specific T cell response should be an issue for future studies.

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