

Blocking Antibodies Induced by Specific Allergy Vaccination Prevent the Activation of CD4⁺ T Cells by Inhibiting Serum-IgE-Facilitated Allergen Presentation

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Allergen-specific CD4⁺ T lymphocytes are activated at extremely low allergen concentrations in vivo as a result of serum-facilitated allergen presentation (S-FAP). It is not clear at present if specific allergy vaccination (SAV) has an effect on this mechanism. Here we show that birch allergen-specific serum-IgE facilitates the presentation of Bet v 1, the major birch pollen allergen, to Bet v 1-specific CD4⁺ T lymphocytes by a factor of >100. This process is CD23 mediated, could be detected in sera from the majority of birch-allergic patients, and was clearly dose dependent. S-FAP of Bet v 1 was inhibited in patients undergoing long-term birch SAV, but not by sera from patients undergoing grass SAV, indicating that birch-specific Abs are involved. This resulted in decreased proliferation and IL-4, IL-5, IL-10, and IFN- γ production of Bet v 1-specific T cells. The inhibition was already noted after 3–9 mo of SAV and could not be solely explained by increased serum levels of birch-specific IgG4. When IgG- and IgA/IgM-containing fractions of long-term SAV sera were used to inhibit S-FAP, only IgG-containing fractions were shown to inhibit S-FAP. These results indicate that blocking IgG Abs induced by SAV inhibits the occurrence of S-FAP at very low allergen concentrations, resulting in significantly higher allergen threshold levels to obtain T cell proliferation and cytokine production and thus allergen-induced late-phase responses. *The Journal of Immunology*, 1999, 163: 2944–2952.

Atopic allergy is characterized by elevated levels of allergen-specific serum-IgE and tissue eosinophilia, two parameters that are responsible for the immediate and late-phase allergic responses, respectively. It is well established that both IgE production and eosinophilia are dependent on the presence and activation of allergen-specific CD4⁺ T lymphocytes with a bias toward the production of Th2 cytokines (1, 2). These Th2-like cells produce IL-4, which induces B lymphocytes to switch to the production of IgE, and IL-5, which plays an important role in the differentiation and maturation of eosinophilic granulocytes. Allergen-specific Th2 cells have been identified in the peripheral blood (1, 2) as well as in affected tissues of allergic patients (3–5), underlining the role that allergen-specific T cells play in atopic allergies.

In addition, atopic allergic patients have been shown to have an increased expression of CD23 on B cells and macrophages (6, 7) and Fc ϵ RI on monocytes and dendritic cells (8, 9). The expression of IgE Fc receptors on APC suggests that they may play a functional role in the presentation of allergens to specific T cells. Indeed, both IgE receptors have been shown to facilitate the presentation of allergens in the presence of specific serum-IgE, resulting in T cell activation at 100- to 1000-fold lower allergen concentrations than are required when control sera are used (10–14). This is a crucial step in the activation of allergen-specific Th2-like cells, because the factual exposure to airborne allergens is extremely

low, i.e., in the range of several micrograms per year. Therefore, inhibition of this mechanism would theoretically result in increasing the allergen concentrations required for in vivo T cell activation and Th2 cytokine production.

Specific immunotherapy with allergen extracts, recently termed therapeutic allergy vaccines in the World Health Organization position paper on allergen immunotherapy (15), has been practiced since its introduction by Noon in 1911 (16). Specific allergy vaccination (SAV)² is currently the only allergy treatment that not only has an antisymptomatic effects but also influences the course of the allergic disease itself (15). The clinical efficacy of SAV is well documented (reviewed in Ref. 15), 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. Most importantly, SAV has been shown to have long-term efficacy (17, 18), prevent the development of new sensitizations (19), and even inhibits the progression from rhinitis to asthma (20).

However, the precise immunological mechanisms underlying SAV are not entirely clear. Several immunological effects have been described. There is 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 (21, 22); a sharp rise in allergen-specific IgG4 Abs (23, 24), and a decreased number of circulating basophils (25). However, specific serum-IgE levels do not decrease after SAV but rather show a transient increase (26). In addition, a shift in the production of T cell cytokines from Th2 to a more moderate Th0 type (27–30), a decreased proliferative response of peripheral T cells (28, 31), an increased production of IL-10 by T cells (32), as well as an increased production of IL-12 (33) and IL-10 (32) by APC has been reported. However, these immunological changes that SAV induces cannot completely

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² Abbreviations used in this paper: SAV, specific allergy vaccination; LPR, late phase responses; S-FAP, serum-facilitated allergen presentation; TCC, T cell clone.

explain the clinical observations that much higher allergen threshold levels are required to obtain a positive late-phase response (LPR). As inhibition of serum-facilitated allergen presentation (S-FAP) would offer a valid explanation of these clinical observations, the present study addresses the question whether SAV interferes with S-FAP, thereby explaining why SAV is clinically efficacious without changes in serum levels of allergen-specific IgE.

Materials and Methods

Abs and reagents

Anti-CD23 mAb MHM6, anti-CD19 mAb HD37, FITC-labeled F(ab')₂ of goat anti-mouse IgG, rabbit anti-human IgG, and rabbit anti-human IgE were obtained from Dako (Glostrup, Denmark). Anti-FcεRI mAb 15-1 was a kind gift of Dr. J. P. Kinet (Boston, MA), anti-CD80 mAb BB1 and anti-CD86 mAb FUN-1 were obtained from PharMingen (San Diego, CA), anti-CD40 mAb 89 was purchased from Immunotech (Marseille, France), and anti-CD3 mAb OKT3 and anti-HLA-DR mAb L243 were obtained from American Type Culture Collection (Manassas, VA).

Sera and IgE and IgG4 measurements

Sera from healthy controls, birch-allergic patients, birch-allergic patients undergoing SAV (birch Alutard SQ, ALK-Abelló, Hørsholm, Denmark), and grass-allergic patients undergoing SAV (grass Alutard SQ) were obtained from our internal serum bank. The duration of SAV is defined as months of therapy after initial up dosing. The study was approved by the local ethics committee. All sera were blinded, and clinical data were not available. Allergen-specific IgE and IgG4 were measured using the Magic Lite SQ specific IgE system (ALK-Abelló) (34).

Fractionation of long-term SAV sera

Two long-term SAV sera (1598 and 1490) were separated into IgG- and IgM/IgA-containing fractions by separating the sera on a γ Bind Plus protein G column (Pharmacia, Uppsala, Sweden). The column was washed with PBS, pH 7.2, followed by 2 ml of serum. The run through was collected, and the IgG bound to the column was eluted using a Pierce elution buffer (Pierce, Rockford, IL). All fractions were extensively dialyzed against PBS, and volumes were adjusted to the starting volume.

The presence of IgM, IgG, and IgA was measured using a human IgG, IgA, and IgM "NL" bind a rid kit according to the manufacturer's description (The Binding Site, Birmingham, U.K.).

Preparation of Bet v 1

Bet v 1 was enriched from an aqueous birch pollen extract as described by Ipsen (35, 36). In brief, size-exclusion chromatography of 150 mg (dry weight) of the pollen extract was performed on a G-75 superfine (Pharmacia) 90 cm × 2 cm² (K15/100; Pharmacia) 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.

Culture conditions

T cells were cultured in RPMI 1640 medium (Life Technologies, Paisley, U.K.) supplemented with 2 mM L-glutamine (Life Technologies), 100 IU/ml of penicillin-streptomycin (Life Technologies), and 5% heat-inactivated, screened human AB+ serum (BioWhittaker, Walkersville, MD) (later referred to as complete medium). All cultures were grown under sterile conditions at 37°C in a humidified atmosphere of 5% CO₂. EBV-transformed B cell lines were cultured in RPMI 1640, 2 mM L-glutamine, 100 IU penicillin-streptomycin, and 10% heat-inactivated FCS (Life Technologies) 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 birch-allergic patient AF, who is clinically allergic to birch pollen (37). The CD4⁺ T cell clone (TCC) AF28 reacted with aa 21–33, CD4⁺ TCC AF19 and AF24 with aa 37–51, and AF line (which contains both CD4⁺ and CD8⁺ T cells) reacted with several T cell epitopes simultaneously.

Preparation of EBV-transformed B cells for facilitated Ag presentation

Serum of birch-allergic patients and control donors was incubated for 1 h at 37°C with serial dilutions of Bet v 1. Then EBV-B cells (irradiated with 5000 rad) were added at a concentration of 3.10⁵/ml to the allergen-complexed serum and incubated for 1 h at 37°C. After incubation, the cells were washed two times in RPMI 1640 and used as APC in T cell stimulation assays.

In blocking experiments with polyclonal anti-IgG or anti-IgE, the sera were incubated with these antisera for 1 h at 37°C before adding Bet v 1. In blocking experiments with anti-CD23, EBV-B cells were incubated for 1 h at 4°C with this Ab before adding the cells to the sera. In experiments in which S-FAP induced by a patient serum was inhibited by SAV sera or fractions thereof, a mixture of the two sera (both at 40%) was preincubated for 1 h at 37°C before B cells were added.

Flow cytometric analysis

Cells were Ficoll-separated, washed twice in Cellwash (Becton Dickinson, San Jose, CA), and labeled on ice for 30 min with mAb diluted in Cellwash. Subsequently, cells were washed three times and incubated with FITC-labeled F(ab')₂ fragments of rabbit anti-mouse Ig (Dako). Cells were washed three times again, and fluorescence was analyzed on a FACScalibur flow cytometer (Becton Dickinson). For each sample, 10,000 cells were analyzed. No gates were set.

Proliferative responses of Bet v 1-specific T cell line and clones

Bet v 1-specific T cells were washed twice in RPMI 1640 medium to remove excess PHA and IL-2. Ag-specific proliferation was assessed by stimulation of the T cells, 2–4 × 10⁴ cells/well, by Ag using irradiated (5000 rad) autologous EBV-transformed B cells as APC (1–2 × 10⁴ 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), 0.5 μCi/well. Proliferation was expressed as mean cpm of [³H]TdR incorporation of triplicate cultures. All experiments shown were performed at least two times with AF line and at least one of the CD4⁺ Bet v 1-specific TCC.

Production of supernatants for cytokine measurements

Bet v 1-specific T cells (2–4 × 10⁴/well) were activated for 24 h in the presence or absence of Bet v 1 in the presence of autologous, irradiated EBV-transformed B cells (1–2 × 10⁴ cells/well) as described above. Supernatants were collected for cytokine measurements after a 24-h culture period.

IL-4, IL-5, IL-10, and IFN-γ ELISA

The presence of IL-4, IL-5, IFN-γ, and IL-10 in the TCC supernatants was measured using the DuoSet (IL-4, IL-5, and IFN-γ) or Predicta (IL-10) ELISA kits according to the manufacturer's description (Genzyme, Cambridge, MA).

Results

Sera containing high levels of birch-specific IgE facilitate allergen presentation to CD4⁺ T cells

To address the role of serum IgE in the presentation of Bet v 1 to specific T cells, two CD4⁺ Bet v 1-specific T cell clones and a polyclonal Bet v 1-specific T cell line from donor AF were studied in an in vitro culture system model using autologous EBV-transformed B cells (CD23⁺, CD19⁺, CD20⁺, HLA-DR⁺, CD40⁺, CD80⁺, CD86⁺, CD3[−], and FcεRI[−]) as APC. This culture system has been used previously to show S-FAP of the house dust mite allergen Der p 2 (10). Six sera containing very high levels of birch-specific IgE and three control sera that did not contain any detectable IgE (Table I) were preincubated with serial dilutions of Bet v 1 for 1 h at 37°C, AF EBV-B cells were added for 1 h at 4°C to prevent fluid phase endocytosis, and the B cells were washed twice to remove any free allergen not complexed with IgE and bound to CD23. T cells were added, and proliferation was measured after a 24-h culture period. Fig. 1 shows that the control sera could not facilitate the presentation of Bet v 1 to the Bet v 1-specific T cells. All sera containing high levels of birch-specific IgE could facilitate

Table I. Specific IgE measurements of sera of birch-allergic patients

Serum No.	Birch-Specific IgE ^a (SU/ml)	Grass-Specific IgE (SU/ml)	Total IgE (IU/ml)
Control Sera			
734	<1	<1	<30
745	<1	<1	<30
981	<1	<1	<30
Patient Sera			
1151	3	182	282
831	4	9	92
1446	6	39	148
1547	14	48	177
1531	23	82	>3,000
1327	50	118	805
1370	80	6	151
920	143	29	>3,000
1372	241	4	262
1463	310	119	>3,000
1374	373	<	279
1008	795	94	571
1464	>800	60	2,826
102	>800	34	19,000
894	>800	290	1,283
1083	>800	94	NT ^b

^a IgE levels present in sera were measured using the Magic Lite system as described in *Materials and Methods*. Values given for specific IgE levels are expressed as standardized units of specific IgE per ml (SU/ml), and total IgE levels are expressed as international units per ml (IU/ml).
^b NT, not tested.

the presentation of Bet v 1 to the T cells, and significant proliferation could be measured at 100 and even at 10 ng/ml, indicating that >100-fold lower allergen concentrations are sufficient to activate Bet v 1-specific T cells in the presence of high levels of specific serum IgE.

S-FAP of Bet v 1 is even more efficient at 37°C

As allergen presentation studied at 4°C may not be relevant to the in vivo situation, similar experiments were performed with B cell incubation at 4°C, 37°C, and 37°C without washing away excess Bet v 1 and serum. As shown in Fig. 2, S-FAP by patient serum occurred at 4°C and, more efficiently, at 37°C. When excess allergen was not washed away after the incubation with APC at 37°C, an even more efficient presentation of Bet v 1 was observed,

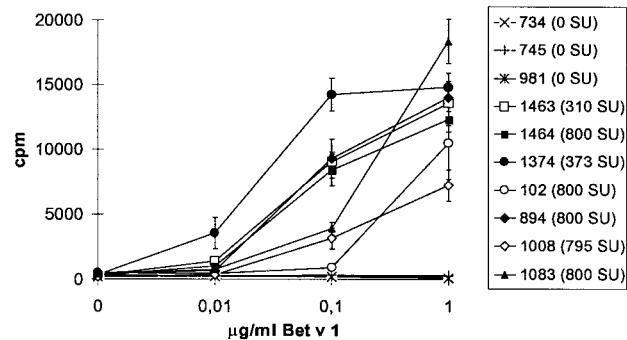


FIGURE 1. Allergen presentation of Bet v 1 is facilitated by patient sera containing high levels of birch-specific IgE. Serial dilutions of Bet v 1 were preincubated with 80% serum from birch-allergic patients (1463, 1464, 1374, 102, 894, 1008, 1083) or control donors (734, 745, 981). AF EBV-B cells were added, incubated for 1 h at 37°C, and were cocultured ($1-2 \times 10^4$ /well) with Bet v 1-specific T cell line AF ($2-4 \times 10^4$ /well) for 24 h, followed by a 16-h [^{3} H]TdR pulse and liquid scintillation counting. Values shown are mean cpm of triplicate cultures. Error bars represent SDs.

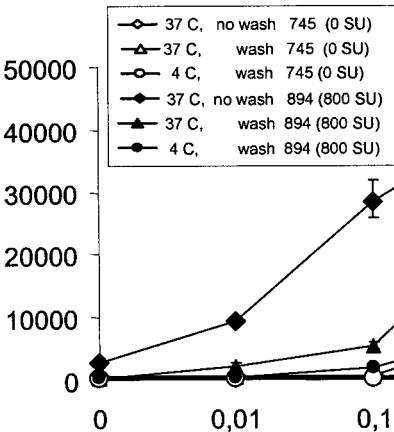


FIGURE 2. S-FAP of Bet v 1 is more effective at 37°C than at 4°C. Serial dilutions of Bet v 1 were preincubated with 80% patient serum 894 or nonallergic control serum 745 for 1 h at 4°C or 37°C. AF EBV-B cells were added and incubated for 1 h at 37°C. The AF EBV-B cells ($1-2 \times 10^4$ /well) were used as APC and were cocultured with Bet v 1-specific T cell line AF ($2-4 \times 10^4$ /well) for 24 h, followed by a 16-h [^{3} H]TdR pulse and liquid scintillation counting. Values shown are mean cpm of triplicate cultures. Error bars represent SDs.

which may be explained by additional allergen presentation via non-IgE-mediated routes. Control sera were not able to induce T cell proliferation when the incubation with APC was performed at 4°C, but could be detected at the highest allergen concentrations when the incubation was performed at 37°C. This is probably the result of Ag uptake via fluid-phase endocytosis. These results indicate that S-FAP occurs very efficiently at physiological temperature, and therefore additional experiments were performed at 37°C, unless stated otherwise.

S-FAP of Bet v 1 can be inhibited by Abs to IgE and CD23, but not by control Abs to IgG or CD19

S-FAP of Bet v 1 occurred when sera containing high levels of birch-specific IgE were used. To show that binding of IgE/allergen

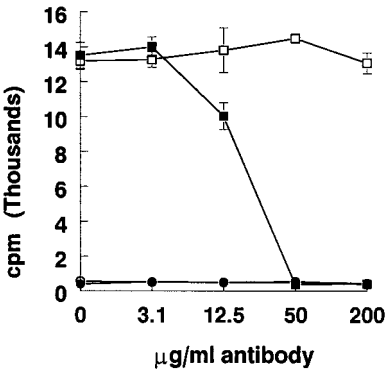


FIGURE 3. S-FAP of Bet v 1 is inhibited by α IgE, but not by α IgG Abs. Bet v 1 (0.1 μ g/ml) was preincubated for 1 h at 37°C with patient serum 1464 (at a final serum concentration of 40%) in the absence or presence of serial dilutions of polyclonal anti-IgE or anti-IgG Abs. Control serum 734 was used as a negative control. AF EBV-B cells were added and incubated for 1 h at 37°C. AF EBV-B cells ($1-2 \times 10^4$ /well) were used as APC and were cocultured with Bet v 1-specific TCC AF24 ($2-4 \times 10^4$ /well) for 24 h, followed by a 16-h [^{3} H]TdR pulse and liquid scintillation counting. Values shown are mean cpm of triplicate cultures. Error bars represent SDs.

Table II. Inhibition of serum IgE-mediated allergen presentation by anti-CD23^a

mAb (μ g/ml)	Patient Serum 1464		Control Serum 734	
	+ Anti-CD19	+ Anti-CD23	+ Anti-CD19	+ Anti-CD23
0	2.5 $\pm$ 0.3	3.7 $\pm$ 0.2	0.3 $\pm$ 0.0	0.3 $\pm$ 0.1
0.6	4.2 $\pm$ 0.4	4.1 $\pm$ 0.7	0.5 $\pm$ 0.1	0.7 $\pm$ 0.1
2.5	2.9 $\pm$ 0.7	3.4 $\pm$ 0.6	0.6 $\pm$ 0.0	0.7 $\pm$ 0.1
10	5.3 $\pm$ 0.2	0.8 $\pm$ 0.1	0.7 $\pm$ 0.1	0.8 $\pm$ 0.1
40	4.5 $\pm$ 0.6	0.9 $\pm$ 0.1	0.5 $\pm$ 0.1	1.0 $\pm$ 0.0

^aBet v 1 (0.1 μ g/ml) was preincubated for 1 h at 37°C with control serum 734 or with birch-allergic patient serum 1464 (>800 SU/ml birch-specific IgE in MagicLite assay) at a serum concentration of 33%. AF EBV-B cells were incubated for 1 h at 4°C with no Ab, with serial dilutions of anti-CD23 mAb MHM6, or with anti-CD19 mAb HD37, followed by two wash steps. The EBV-B cells were added to the preincubated serum fractions for 1 h at 37°C, resulting in a final cell concentration of 4×10^6 /ml and a serum concentration of 16.7%, followed by two washes to remove excess allergen. The EBV-B cells ($1-2 \times 10^4$ /well) were used as APC and were cocultured with Bet v 1-specific TCC AF24 ($2-4 \times 10^4$ /well) for 24 h, followed by a 16-h [³H]TdR pulse and liquid scintillation counting. Values shown are mean cpm ($\times 10^{-3}$) of triplicate cultures $\pm$ SD.

complexes to CD23 mediated to this effect, two sets of experiments were performed. First, patient and control sera were preincubated for 1 h at 37°C with serial dilutions of polyclonal IgE- or IgG-specific rabbit antiserum before allergen was added. Fig. 3 clearly shows that anti-IgE Abs completely inhibited the serum-facilitated presentation of Bet v 1, whereas anti-IgG Abs had no effect. To evaluate the role of CD23 in our culture model, experiments were performed by blocking CD23 or CD19 as a control B cell marker by preincubating the B cells for 1 h at 4°C with anti-CD23 or anti-CD19 mAb. Table II shows that preincubation of AF EBV-B cells with anti-CD23 mAb, but not anti-CD19 mAb, prevented the occurrence of S-FAP of Bet v 1 to Bet v 1-specific T cells. These experiments demonstrate that S-FAP of Bet v 1 is IgE dependent and is mediated via binding to CD23.

S-FAP of Bet v 1 is dependent on the level of birch-specific IgE Abs

To further study the relevance of S-FAP in birch-allergic patients, sera were collected from a panel of patients with different levels of

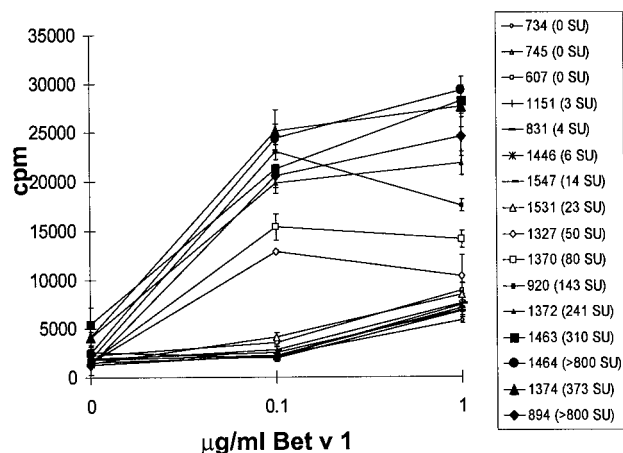


FIGURE 4. S-FAP is detectable in sera containing ≥ 50 SU/ml birch-specific IgE. Bet v 1 was preincubated with sera from birch-allergic patients sera (at a final serum concentration of 80%) containing varying amounts of birch-specific IgE for 1 h at 37°C. AF EBV-B cells were added and incubated for 1 h at 37°C. The AF EBV-B cells were cocultured with Bet v 1-specific AF cell line ($2-4 \times 10^4$ /well) for 24 h, followed by a 16-h [³H]TdR pulse and liquid scintillation counting. Values shown are mean cpm of triplicate cultures. Error bars represent SDs.

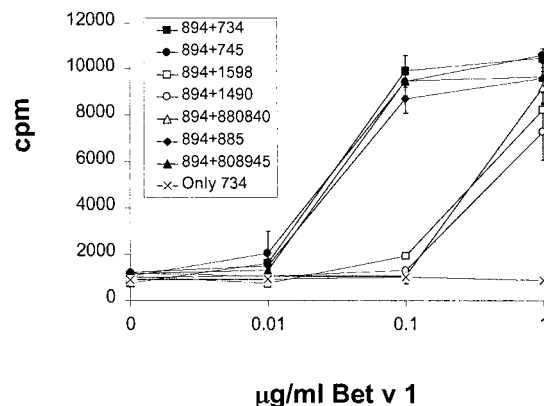


FIGURE 5. S-FAP of Bet v 1 is inhibited by birch SAV sera, but not by grass SAV sera. Bet v 1 (0.1 μ g/ml) was preincubated for 1 h at 37°C with patient serum 894 at a serum concentration of 40% in the presence of nonallergic control sera (734, $\blacksquare$; 745, $\bullet$), long-term birch SAV sera (1598, $\square$; 1490, $\circ$; 808840, $\triangle$), or long-term grass SAV sera (885, closed lozenge; 808945, $\blacktriangle$) all at 40%. Control serum 734 (at 40%) without patient serum was used as a negative control (stippled line). AF EBV-B cells were added and incubated for 1 h at 37°C. The EBV-B cells ($1-2 \times 10^4$ /well) were used as APC and were cocultured with Bet v 1-specific TCC AF19 ($2-4 \times 10^4$ /well) for 24 h, followed by a 16-h [³H]TdR pulse and liquid scintillation counting. Values shown are mean cpm of triplicate cultures. Error bars represent SDs.

birch-specific IgE (Table I). The sera that were used initially were sera containing >300 SU of birch-specific IgE, representing only a small percentage of all birch-allergic patients. As is shown in Fig. 4, S-FAP of Bet v 1 was observed in a serum-IgE-dependent fashion. Sera containing >100 SU birch-specific IgE were very efficient in mediating presentation of Bet v 1, but even sera containing 50–100 SU were able to mediate the effect. All other sera with <50 SU birch-specific IgE tested could not facilitate the presentation of Bet v 1. This indicates that the effect is relevant to the majority of clinically allergic patients.

Sera from patients undergoing birch SAV but not sera from patients undergoing grass SAV specifically inhibit S-FAP of Bet v 1 to specific T cells

As sera from patients undergoing SAV contained high levels of allergen-specific IgG4 Abs, we wanted to address the question whether sera from patients undergoing birch SAV could inhibit the observed facilitated presentation of Bet v 1. To study this, Bet v 1 was preincubated for 1 h at 37°C with a patient serum containing high levels of birch-specific IgE in the presence of medium, non-allergic control sera, long-term birch SAV sera, or long-term grass SAV sera. Fig. 5 shows that all three long-term birch SAV sera inhibited S-FAP of Bet v 1 to the Bet v 1-specific T cells. Similar results were obtained when the experiments were performed at 4°C (data not shown). The effect was specific for birch SAV, because neither control sera nor long-term grass SAV sera could inhibit the effect. This effect was noted in all such experiments. In addition, IgE-mediated allergen presentation of the grass allergen Phl p 5 to a Phl p 5-specific T cell clone could be inhibited with long-term grass SAV but not birch SAV sera, further supporting the relevance of this finding (data not shown).

The inhibition of S-FAP of Bet v 1 by birch SAV sera inhibits T cell proliferation, as well as the production of T cell cytokines IL-4, IL-5, IL-10, and IFN- γ

To address the effect of the inhibition of T cell activation with SAV sera on cytokine production, supernatants were removed

Table III. Inhibition of S-FAP measured as T cell proliferation and cytokine production by birch SAV sera

T Cell	Ag/Incubation	cpm ^a ($\times 10^{-3}$)	IL-5 (pg/ml)	IL-4 (pg/ml)	IFN- γ (pg/ml)	IL-10 (pg/ml)
AF28	No Ag	0.4	<	<	<	<
	Bet v 1 in well (10 μ g/ml)	4.3	>3000	1746	<	<
	+ 920 + 734 (control serum)	6.6	798	228	<	<
	+ 920 + 1598 (>30m birch SAV)	1.3	<	<	<	<
0.01 μ g/ml	+ 920 + 885 (>30m grass SAV)	7.4	1876	299	<	175
	No Ag	1.0	<	<	<	<
	Bet v 1 in well (10 μ g/ml)	35.6	>3000	416	308	1010
	+ 920 + 734 (control serum)	30.6	1907	72	<	124
AF line	+ 920 + 1598 (>30m birch SAV)	6.9	93	<	<	<
	+ 920 + 885 (>30m grass SAV)	24.7	1368	79	<	121

^a Bet v 1 (0.01 μ g/ml) was preincubated for 1 h at 37°C with patient serum 920 in the presence of nonallergic control serum 734, long-term birch SAV serum 1598, or long-term grass SAV serum. AF EBV-B cells were added to a final concentration of 2.5×10^6 /ml and incubated for 1 h at 37°C, followed by washing away excess Ab and allergen. The EBV-B cells ($1-2 \times 10^4$ /well) were used as APC and were cocultured with Bet v 1-specific T cell line AF ($2-4 \times 10^4$ /well). After 24 h, supernatant was removed for cytokine measurements, and the cells were pulsed for 16 h with [³H]TdR and liquid scintillation counting.

from cultures that had been set up under S-FAP conditions with a control serum, long-term birch SAV serum, and a long-term grass SAV serum. Table III shows that not only T cell proliferation, but also the production of the cytokines IL-4, IL-5, IL-10, and IFN- γ , was inhibited by the presence of birch SAV serum, but not by the presence of the control sera.

Inhibition of S-FAP of Bet v 1 occurs rapidly after starting birch SAV treatment

Clinical efficacy of SAV is already noticeable after short-term SAV. To evaluate if the inhibitory effect of birch SAV sera on presentation of Bet v 1 could be demonstrated with short-term

birch SAV sera, inhibition experiments were performed with a range of birch SAV sera (shown in Table IV). With these sera, inhibition could only be demonstrated after >30 mo of birch SAV (data not shown). However, inhibition experiments are performed by mixing an IgE-containing serum with a SAV serum, which may not completely represent the in vivo situation.

Therefore, control sera, sera from birch-allergic patients receiving no treatment, and birch-allergic patients receiving birch SAV were matched for birch-specific serum IgE, and their ability to facilitate the presentation of Bet v 1 to specific T cells was measured in a direct S-FAP assay (Fig. 6A for sera containing 100–300 SU/ml birch-specific IgE and Fig. 6B for sera containing 50–100

Table IV. Measurement of birch and grass allergen-specific IgG4 and IgE

Serum	Birch IgE (SU/ml)	Grass IgE (SU/ml)	Total IgE (IU/ml)	SAV (mo)	Birch IgG4 (RLU)	Grass IgG4 (RLU)
Controls						
734	<	<	<30	No SAV	3,369	2,236
745	<	<	<30	No SAV	7,510	5,636
Birch allergics						
1327	50	118	805	No SAV	17,732	16,771
1370	80	6	151	No SAV	13,984	28,065
1568	56	3	880	No SAV	3,871	1,654
1372	241	4	262	No SAV	NT ^a	NT
1451	164	2	66	No SAV	6,983	3,577
1334	133	29	218	No SAV	11,975	395,914
920	143	29	>3,000	No SAV	18,130	10,621
Birch SAV						
1430	61	<	34	3–9 ^b	293,792	437,325
809218	85	209	NT	3–9 ^c	271,105	654,830
808926	123	445	251	9–18 ^b	114,544	367,286
1459	157	125	1,035	9–18 ^b	211,815	477,977
1293	108	<	30	9–18	190,292	8,643
809622	203	<	225	18–30	254,874	25,180
809038	140	166	340	18–30 ^c	5,035	416,457
939	130	85	310	18–30 ^c	141,947	437,288
809978	62	<	79	18–30	165,063	5,133
1598	67	<	29	>30	379,707	3,933
1490	88	<	124	>30	4,643	1,874
1713	113	<	73	>30	102,049	6,254
809652	280	5	252	>30 ^c	187,027	191,878
808840	114	<	NT	>30	189,814	3,387
Grass SAV						
885	<	150	871	>30 ^d	6,046	399,663
808954	4	137	NT	>30 ^d	10,272	410,198

^a NT, not tested.

^b Birch, grass, and other SAV.

^c Birch and grass SAV.

^d Grass, but not birch, SAV.

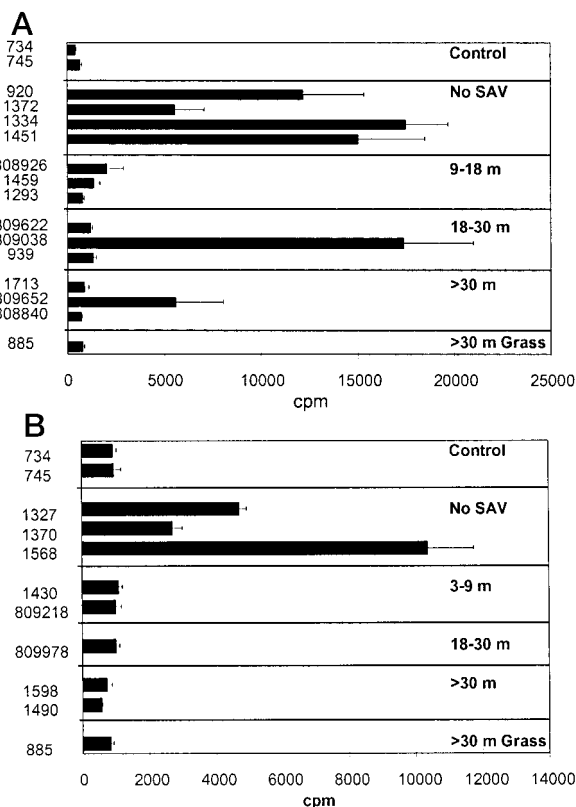


FIGURE 6. Inhibition of S-FAP by SAV sera occurs rapidly after the start of treatment. Bet v 1 (0.01 $\mu\text{g/ml}$) was preincubated for 1 h at 37°C with sera from birch-allergic patients, nonallergic control sera, birch SAV sera, or grass SAV sera (all at 80%). The birch-specific serum IgE levels in the birch-allergic sera and the birch SAV sera were matched (i.e., 100–300 SU/ml birch-specific IgE (A) or 50–100 SU/ml birch-specific IgE (B)). AF EBV-B cells were added and incubated for 1 h at 37°C. The EBV-B cells ($1-2 \times 10^4$ /well) were used as APC and were cocultured with Bet v 1-specific T cell line AF ($2-4 \times 10^4$ /well) for 24 h, followed by a 16-h [^3H]TdR pulse and liquid scintillation counting. Values shown are mean cpm of triplicate cultures. Error bars represent SDs.

SU/ml birch-specific IgE). These experiments clearly demonstrated that sera from untreated patients facilitate Bet v 1 presentation and that sera from the majority of patients undergoing birch SAV have a decreased ability to do so. This effect was already noted after 3–9 mo of birch SAV. The fact that some patients did not have a decreased ability to mediate S-FAP (809038, 809652) may indicate that these patients did not have optimal clinical efficacy. In line with the data presented in Table III, IL-5 production was also decreased in culture supernatants of these experiments, with the exception of the same two sera that did not have a decreased ability to mediate S-FAP (data not shown).

Inhibition of S-FAP after SAV is not solely dependent on serum levels of allergen-specific IgG4

As serum levels of allergen-specific IgG4 have been documented to increase after SAV, birch-specific IgG4 Ab levels were determined in the SAV sera (Table IV). Subjects were divided into several groups: nonallergic controls, birch-allergic controls that did not undergo SAV, birch-allergic patients that underwent 3–9 mo of SAV, 9–18 mo SAV, 18–30 mo of SAV, and >30 mo SAV. In addition, sera from grass allergic patients that had no sensitization to birch and had >30 mo of grass SAV were included as control sera. The levels of birch- and grass-specific IgE and IgG4 were measured in all these sera. Table IV shows that nonallergic

Table V. Fractionation of long-term birch SAV sera^a

	IgG ($\mu\text{g/ml}$)	IgA ($\mu\text{g/ml}$)	IgM ($\mu\text{g/ml}$)
1598			
Serum	14,000	5,500	400
Run through	0	5,700	400
Eluate	9,900	0	0
1490			
Serum	13,500	6,500	400
Run through	0	6,500	400
Eluate	9,800	0	0

^a Two long-term birch SAV sera (1598 and 1490) were fractionated on a protein G-Sepharose column. The eluate and run through fractions were adjusted to the original volume of the serum, and contents of IgG, IgA, and IgM were measured (see *Materials and Methods*).

controls and allergic patients that did not undergo SAV had very low levels of birch- or grass-specific IgG4. In contrast, sera from patients undergoing birch- or grass-specific SAV had high levels of specific IgG4 Abs, independent on the duration of treatment. Only one serum long-term birch SAV (serum 1490) did not have high serum levels of birch-specific IgG4. However, this serum was as potent as the other SAV sera in inhibiting S-FAP (see Figs. 5 and 6). Another serum (809652) did have increased birch allergen-specific IgG4 but showed no inhibition of S-FAP (Table IV, Fig. 6A). In addition, as stated above, we have only been able to measure the inhibition of S-FAP in indirect assays with long-term SAV sera. Short-term SAV sera that contained similar or even higher levels of birch-specific IgG4 could not inhibit S-FAP (data not shown), strongly indicating that the inhibition of S-FAP noted cannot solely be explained by increased serum-levels of birch allergen-specific IgG4.

The inhibition of S-FAP after SAV is mediated by blocking IgG Abs

To further investigate which type of Ab mediates the blocking of S-FAP after SAV, we separated two long-term SAV sera on a protein G column. As Table V shows, all IgG Abs were present in the eluate of the column, and all IgA and IgM Abs were present in the

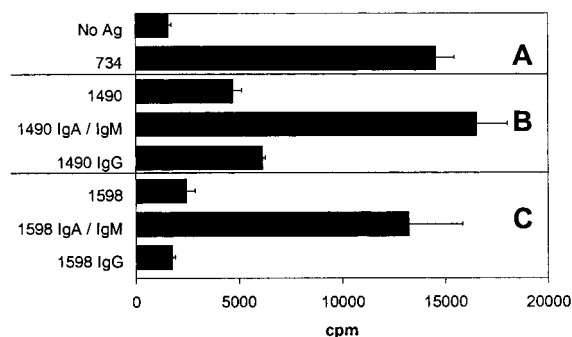


FIGURE 7. IgG but not IgA or IgM present in SAV sera inhibit S-FAP. Bet v 1 (0.01 $\mu\text{g/ml}$) was preincubated for 1 h at 37°C with patient serum 1372 at a serum concentration of 40% in the presence of nonallergic control serum 734, long-term birch SAV sera 1598 and 1490, or fractions of these sera containing IgG or IgA and IgM (all at 40%). Serum 1372 (at 40%) without Ag was used as a negative control. AF EBV-B cells were added to a final concentration of 1.5×10^6 /ml for 1 h at 37°C, followed by washing away excess serum and allergen. The EBV-B cells ($1-2 \times 10^4$ /well) were used as APC and were cocultured with Bet v 1-specific TCC AF19 ($2-4 \times 10^4$ /well) for 24 h, followed by a 16-h [^3H]TdR pulse and liquid scintillation counting. Values shown are mean cpm of triplicate cultures. Error bars represent SDs.

run through. When the sera and the serum fractions were tested for their ability to inhibit S-FAP, only unfractionated sera and the fractions containing IgG inhibited S-FAP (Fig. 7), demonstrating that blocking IgG Abs but not IgA or IgM Abs inhibit S-FAP. This is supported by our observation that rabbit anti-Bet v 1 antiserum, as well as murine Bet v 1-specific monoclonal IgG Abs, also inhibit S-FAP (data not shown).

Discussion

The presentation of extremely low doses of allergen to CD4⁺ T lymphocytes is greatly enhanced by IgE-dependent Ag focussing to CD23 or FcεRI. Here, we demonstrate that this process is inhibited by blocking allergen-specific IgG Abs that are induced by SAV. As a result of this, increased doses of allergens are required to induce T cell proliferation and cytokine production *in vivo*.

An increasing body of evidence indicates that S-FAP is a mechanism that occurs *in vivo*. Atopic allergic individuals have been shown to have increased expression of CD23 on B cells and macrophages (6, 7) and FcεRI on monocytes and dendritic cells (8, 9). Both receptors have been shown to be involved in S-FAP *in vitro* (10–14). *In vivo* Th2 responses and specific IgG1 and IgE production in mice are enhanced by allergen-IgE complexes (38, 39) or by allergen-anti-CD23 mAb conjugates (40), whereas CD23 knockout mice lack this ability (41).

In addition, clinical trials with a neutralizing anti-IgE mAb have shown that anti-IgE mAb inhibits bronchial LPR and the number of eosinophils in sputum of allergic asthma patients (42), strongly suggesting that IgE is crucial for the development of LPR (43, 44) and that inhibition of this mechanism results in decreased late-phase allergic reactions. Similarly, 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, and the same effect is observed in CD23 knockout mice (45).

All these findings strongly suggest that IgE, as well as IgE receptors, plays an important role in the *in vivo* activation of CD4⁺ T cells, the regulation of humoral responses, and most importantly in late-phase allergic responses.

S-FAP of Bet v 1, the major birch pollen allergen, was shown to occur at both 4°C and 37°C and was dependent on the levels of birch-specific IgE present in the sera. The effect was demonstrated to occur with sera that contained >50 SU birch-specific IgE, indicating that the effect is relevant for the majority of patients undergoing SAV. The local production of allergen-specific IgE in the nasal mucosa of grass pollen-allergic patients (46) indicates that allergens entering the nasal and possibly also the bronchial mucosa may indeed be captured in the target organ by allergen-specific IgE and be focussed to CD23⁺ or FcεRI⁺ APC.

S-FAP induced by patient sera containing >300 SU birch-specific IgE could be inhibited by sera from patients undergoing long-term birch SAV, resulting in a strongly reduced proliferation of Bet v 1-specific CD4⁺ T cells. In addition, the production of the allergy-associated type 2 cytokines IL-4 and IL-5 was strongly inhibited, as was the production of the cytokines IL-10 and IFN-γ.

The effect of this inhibition was allergen specific as it was shown that sera from patients undergoing long-term grass SAV could not inhibit the effect on Bet v 1-specific T cells.

Additional inhibition experiments showed that S-FAP of Bet v 1 could also not be inhibited by a combination of long-term grass SAV sera with grass extract (data not shown), thereby excluding the possibility that IgG-allergen complexes induce an inhibitory signal in B lymphocytes after engagement of FcγRIIb1, as has been shown in other models (47).

Furthermore, when sera from untreated birch-allergic patients and patients undergoing birch SAV that were matched for birch-specific IgE levels were compared in their ability to induce S-FAP, sera from the untreated patients could induce proliferation of allergen-specific T cells at low allergen concentrations, whereas this ability was inhibited in most sera from patients undergoing birch SAV. This further substantiates that SAV-treated patients may require higher allergen levels to activate allergen-specific T cells *in vivo*. The inhibition was not seen in all patients undergoing birch SAV. This may indicate that these patients did not have an optimal clinical response to SAV and that the effect may correlate with the clinical response to treatment. As clinical data of the patients were not available, we are currently testing sera from a placebo-controlled double blind birch SAV study to address this question.

One of the long term-birch SAV sera that inhibited S-FAP did not contain birch-specific IgG4 (serum 1490), and only one of the two sera that did not show a clear inhibition of S-FAP had no high birch-specific IgG4 levels (serum 809038 and 809652). Therefore, the inhibition of S-FAP after SAV could not completely be explained by levels of birch-specific IgG4 induced by birch SAV, which may explain why the rise in specific IgG4 Abs after SAV does not correlate with clinical efficacy (15). However, our data show that serum IgG, and not other Ab classes, mediates the inhibition of S-FAP (Table V and Fig. 7). This indicates that the induction of blocking allergen-specific IgG Abs including IgG4, rather than IgG4 Abs alone, prevent the occurrence of S-FAP. These data are supported by the observation that rabbit anti-Bet v 1 antiserum, as well as Bet v 1-specific monoclonal IgG Abs, can also inhibit S-FAP (data not shown).

Late-phase allergic responses have been shown to be dependent on the presence and activation of CD4⁺ T lymphocytes (48–52), eosinophils (50, 51), and factors involved in eosinophil differentiation such as IL-5 (43, 53, 54). Allergen threshold levels for immediate hypersensitivity responses and LPR are strongly increased after SAV (reviewed in Refs. 15 and 55). This may be explained by our observation that sera from patients undergoing SAV have a decreased ability to activate allergen-specific CD4⁺ T cells via S-FAP.

Based on these observations, we would like to propose the following working mechanism of SAV: Initially, SAV induces the production of IL-12 and IL-10 by APC (32, 33). This results in a shift in the production of cytokine-producing CD4⁺ T cells toward the Th0 phenotype (27–30). Both the production of more Th0-like cytokines and IL-10 (32) induce the production of all subclasses of allergen-specific IgG, including IgG4 (32, 56–58).

When these Abs reach sufficiently high levels or affinities to compete with the binding of IgE to allergens (which according to Table IV and Fig. 6 may already occur after 3 mo of SAV), IgE-mediated allergen presentation to CD4⁺ T cells is inhibited at low allergen concentrations, and even histamine release from mast cells and basophils may be inhibited via a similar process (59). Because LPR are mediated by the costimulation-dependent activation and cytokine production by CD4⁺ T lymphocytes (48–52), this results in a much lower LPR as the result of a much higher allergen threshold for T cell activation in the presence of unchanged levels of allergen-specific IgE in the serum of patients undergoing birch SAV.

In addition, it has also been demonstrated that CD23 interacts with CD11b and CD11c on monocytes (60), which results in an increased production of proinflammatory cytokines. Binding of IgE to CD23 inhibits the production of these factors, which include Th1-biasing cytokines. An inhibition of IgE binding to CD23 by SAV as shown in this paper may therefore result in an enhanced production of Th1-biasing cytokines by APC, which results in a

feedback inhibition of Th2 responses. This is accompanied by a shift in the use of B cells to the use of dendritic cells or macrophages as APC, which may also promote Th1, rather than Th2, development.

Thus, the results shown here provide evidence that links many of the previous observations on the mechanisms of SAV. Future research should be focused on the further characterization of the inhibitory allergen-specific IgG responses induced by SAV, as well as on studies that can correlate the observed inhibition of IgE-mediated allergen presentation with clinical efficacy of SAV treatment.

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References

- Wierenga, E. A., M. Snoek, C. J. de Groot, I. Chrétien, J. D. Bos, H. M. Jansen, and M. L. Kapsenberg. 1990. Evidence for compartmentalization of functional subsets of CD4⁺ T lymphocytes in atopic patients. *J. Immunol.* 144:4651.
- Parronchi, P., D. Macchia, M. Piccinni, P. Biswas, C. Simonelli, E. Maggi, M. Ricci, A. A. Ansari, and S. Romagnani. 1991. Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. *Proc. Natl. Acad. Sci. USA* 88:4538.
- van der Heijden, F. L., E. A. Wierenga, J. D. Bos, and M. L. Kapsenberg. 1991. High frequency of IL-4-producing CD4⁺ allergen-specific T lymphocytes in atopic dermatitis lesional skin. *J. Invest. Dermatol.* 97:389.
- Robinson, D. S., Q. Hamid, S. Ying, A. Tscopoulos, J. Barkans, A. M. Bentley, C. Corrigan, S. R. Durham, and A. B. Kay. 1992. Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N. Engl. J. Med.* 326:298.
- Del Prete, G. F., M. De Carli, M. M. D'Elia, P. Maestrelli, M. Ricci, L. Fabbri, and S. Romagnani. 1993. Allergen exposure induces the activation of allergen-specific Th2 cells in the airway mucosa of patients with allergic respiratory disorders. *Eur. J. Immunol.* 23:1445.
- Bujankowski-Weber, J., B. Brings, I. Knoller, T. Pfeil, and W. König. 1989. Expression of low affinity receptor for IgE (FcεRI, CD23) and IgE-BF (soluble CD23) release by lymphoblastoid B-cell line RPMI-8866 and human peripheral lymphocytes of normal and atopic donors. *Immunology* 66:505.
- Takigawa, M., T. Tamamori, D. Horiguchi, T. Sakamoto, M. Yamada, A. Yoshioka, K. Toda, S. Imamura, and J. Yodo. 1991. Fcε receptor II/CD23-positive lymphocytes in atopic dermatitis. I. The proportion of Fc expression of the α-subunit of the high affinity immunoglobulin E receptor (FcεRI-α). *Clin. Exp. Allergy* 26:648.
- Maurer, D., E. Fiebiger, B. Reininger, B. Wolff-Winiski, M. H. Jouvin, O. Kilgus, J. P. Kinet, and G. Stingl. 1994. Expression of functional high affinity immunoglobulin E receptors (FcεRI) on monocytes of atopic individuals. *J. Exp. Med.* 179:745.
- Tunon-de-Lara, J. M., A. E. Redington, P. Bradding, M. K. Church, J. A. Hartley, A. E. Semper, and S. T. Holgate. 1996. Dendritic cells in normal and asthmatic airways: expression of the α-subunit of the high affinity immunoglobulin E receptor (FcεRI-α). *Clin. Exp. Allergy* 26:648.
- van der Heijden, F. L., R. J. J. van Neerven, M. van Katwijk, J. D. Bos, and M. L. Kapsenberg. 1993. Serum IgE-facilitated allergen presentation in allergic disease. *J. Immunol.* 150:3643.
- Pirron, U., T. Schlunck, J. C. Prinz, and E. P. Rieber. 1990. IgE-dependent antigen focussing by human B lymphocytes is mediated by the low-affinity receptor for IgE. *Eur. J. Immunol.* 20:1547.
- Kehry, M. R., and L. C. Yamashita. 1989. Low-affinity IgE receptor (CD23) function on mouse B cells: role in IgE dependent antigen focussing. *Proc. Natl. Acad. Sci. USA* 86:7556.
- Maurer, D., C. Ebner, B. Reininger, E. Fiebiger, D. Kraft, J. P. Kinet, and G. Stingl. 1995. The high affinity IgE receptor (FcεRI) mediates IgE-dependent allergen presentation. *J. Immunol.* 154:6285.
- Mudde, G. C., F. C. van Reijssen, G. J. Boland, G. C. de Gast, P. L. B. Bruijnzeel, and C. A. F. M. Bruijnzeel-Koomen. 1990. Allergen presentation by epidermal Langerhans' cells from patients with atopic dermatitis is mediated by IgE. *Immunology* 69:335.
- Bousquet, J., R. F. Lockey, H. J. Malling, eds. 1998. WHO position paper. Allergen immunotherapy: therapeutic vaccines for allergic diseases. *Allergy* 53(Suppl.):1.
- Noon, L. 1911. Prophylactic inoculation against hay fever. *Lancet* 1:1572.
- Jacobsen, L., B. Nüchel Petersen, J. Å. Wihl, H. Løwenstein, and H. Ipsen. 1997. Immunotherapy with partially purified and standardized tree pollen extracts IV: results from long-term (6-year) follow-up. *Allergy* 52:914.
- Walker, S. M., V. A. Varney, M. Gaga, M. R. Jacobson, and S. R. Durham. 1995. Grass pollen immunotherapy: efficacy and safety during a 4-year follow-up study. *Allergy* 50:405.
- Des Roches, A., L. Paradis, J. L. Menardo, S. Bouges, J. P. Daures, and J. Bousquet. 1997. Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. VI. Specific immunotherapy prevents the onset of new sensitizations in children. *J. Allergy Clin. Immunol.* 99:450.
- Jacobsen, L., S. Dreborg, H. A. Ferduosi, C. Möller, E. Valovirta, U. Wahn, B. Niggemann, S. Sparholt, and H. Løwenstein. 1998. Prevention of asthma by specific immunotherapy (the PAT study): five year follow up. *Allergy* 53(Suppl.43):169 (Abstr.).
- Durham, S. R., S. Ying, V. A. Varney, M. R. Jacobson, R. M. Sudderick, I. S. Mackay, A. B. Kay, and Q. A. Hamid. 1996. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4⁺ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-γ. *J. Allergy Clin. Immunol.* 97:1356.
- Rak, S., A. Björnson, L. Håkanson, S. Sörenson, and P. Venge. 1991. The effect of immunotherapy on eosinophil accumulation and production of eosinophil chemotactic activity in the lung of subjects with asthma during natural pollen exposure. *J. Allergy Clin. Immunol.* 88:878.
- Witteham, A. M., S. O. Stapel, D. H. Sjamsoedin, H. M. Jansen, and R. C. Aalberse. 1996. Fel d 1-specific IgG antibodies induced by natural exposure have blocking activity in skin tests. *Int. Arch. Allergy Immunol.* 109:369.
- Golden, D. B., D. A. Meyers, A. Kagey-Sobotka, M. D. Valentine, and L. M. Lichtenstein. 1982. Clinical relevance of the venom-specific immunoglobulin G antibody level during immunotherapy. *J. Allergy Clin. Immunol.* 69:489.
- Jutel, M., U. R. Müller, M. Fricker, S. Rihs, W. J. Pichler, and C. Dahinden. 1996. Influence of bee venom immunotherapy on degranulation and leukotriene generation in human blood basophils. *Clin. Exp. Allergy* 26:1112.
- Lichtenstein, L., K. Ishizaka, P. Norman, A. Sobotka, and B. Hill. 1973. IgE antibody measurements in ragweed hay fever: relationship to clinical severity and the results of immunotherapy. *J. Clin. Invest.* 52:472.
- Varney, V. A., Q. A. Hamid, M. Gaga, S. Ying, M. Jacobson, A. J. Frew, A. B. Kay, and S. R. Durham. 1993. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late phase cutaneous responses. *J. Clin. Invest.* 92:644.
- Ebner, C., U. Siemann, B. Bohle, M. Willheim, U. Wiedermann, S. Schenk, F. Klotz, H. Ebner, D. Kraft, and O. Scheiner. 1997. Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from Th2 to Th1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. *Clin. Exp. Allergy* 27:1007.
- Jutel, M., W. J. Pichler, D. Skrbic, A. Urwyler, C. Dahinden, and U. R. Müller. 1995. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-γ secretion in specific allergen stimulated T cell cultures. *J. Immunol.* 154:4187.
- Secrist, H., C. J. Chelen, Y. Wen, J. D. Marshall, and D. T. Umetsu. 1993. Allergen immunotherapy decreases interleukin 4 production in CD4⁺ T cells from allergic individuals. *J. Exp. Med.* 178:2123.
- Akdis, C. A., M. Akdis, T. Blesken, D. Wymann, S. S. Afkan, U. Müller, and K. Blaser. 1996. Epitope-specific T cell tolerance to phospholipase A2 in bee venom immunotherapy and recovery by IL-2 and IL-15 in vitro. *J. Clin. Invest.* 98:1671.
- Akdis, C. A., T. Blesken, M. Akdis, B. Wuthrich, and K. Blaser. 1998. Role of interleukin 10 in specific immunotherapy. *J. Clin. Invest.* 102:98.
- Hamid, Q. A., E. Schotman, M. R. Jacobson, S. M. Walker, and S. R. Durham. 1997. Increases in IL-12 messenger RNA⁺ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J. Allergy Clin. Immunol.* 99:254.
- Woodhead, J. S., and I. Weeks. 1989. Magic Lite design and development. *J. Biolumin. Chemilumin.* 4:611.
- Ipsen, H., and O. C. Hansen. 1991. The NH₂-terminal amino acid sequence of the immunologically partial identical major allergens of alder (*Alnus glutinosa*) Aln g 1, birch (*Betula verrucosa*) Bet v 1, hornbeam (*Carpinus betulus*) Car b 1 and oak (*Quercus alba*) Que a 1 pollens. *Mol. Immunol.* 28:1279.
- Ipsen, H., and H. Løwenstein. 1983. Isolation and immunochemical characterization of the major allergen of birch pollen (*Betula verrucosa*). *J. Allergy Clin. Immunol.* 72:150.
- Sparholt, S. H., J. N. Larsen, H. Ipsen, C. Schou, and R. J. J. van Neerven. 1997. Cross-reactivity and T cell epitope-specificity of Bet v 1-specific T cells suggest the involvement of multiple isoallergens in the sensitization to birch pollen. *Clin. exp. Allergy* 27:932.
- Heyman, B., L. Tianmin, and S. Gustavsson. 1993. In vivo enhancement of the specific antibody response via the low-affinity receptor for IgE. *Eur. J. Immunol.* 23:1739.
- Oshiba, A., E. Hamelmann, A. Haczku, K. Takeda, D. H. Conrad, H. Kikutani, and E. W. Gelfand. 1997. Modulation of antigen-induced B and T cell responses by antigen-specific IgE antibodies. *J. Immunol.* 159:4056.
- Squire, C. M., E. J. Studer, A. Lees, F. D. Finkelman, and D. H. Conrad. 1994. Antigen presentation is enhanced by targeting antigen to the FcεRII by antigen-anti-FcεRII conjugates. *J. Immunol.* 152:4388.
- Fujiwara, H., H. Kikutani, S. Suematsu, T. Naka, K. Yoshida, T. Tanaka, M. Suemura, N. Matsumoto, S. Kojima, et al. 1994. The absence of IgE antibody-mediated augmentation of immune responses in CD23-deficient mice. *Proc. Natl. Acad. Sci. USA* 91:6835.
- Fahy, J. V., H. E. Fleming, H. H. Wong, J. T. Liu, J. Q. Su, J. Reimann, R. B. Fick, and H. A. Boushey. 1997. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am. J. Respir. Crit. Care Med.* 155:1828.
- Hamelmann, E., A. Oshiba, J. Schwarze, K. Bradley, J. Loader, G. L. Larsen, and E. W. Gelfand. 1997. Allergen-specific IgE and IL-5 are essential for the development of airway hyper-responsiveness. *Am. J. Resp. Cell. Mol. Biol.* 16:674.

44. Oshiba, A., E. Hamelmann, K. Takeda, K. L. Bradley, J. Loader, G. Larsen, and E. W. Gelfand. 1996. Passive transfer of immediate hypersensitivity and airway hyper-responsiveness by allergen-specific immunoglobulin (Ig) E and IgG1 in mice. *J. Clin. Invest.* 97:1398.
45. Coyle, A. J., K. Wagner, C. Bertrand, S. Tsuyuki, J. Bews, and C. Heusser. 1996. Central role of immunoglobulin-E in the induction of lung eosinophil infiltration and T helper 2 cell cytokine production: inhibition by a non-anaphylactogenic anti-IgE antibody. *J. Exp. Med.* 183:1303.
46. Durham, S. R., H. J. Gould, C. P. Thienes, M. R. Jacobson, K. Masuyama, S. Rak, O. Lowhagen, E. Schotman, L. Cameron, and Q. A. Hamid. 1997. Expression of ϵ germ-line gene transcripts and mRNA for the ϵ heavy chain of IgE in nasal B cells and the effects of topical corticosteroid. *Eur. J. Immunol.* 27:2899.
47. Minskoff, S. A., K. Matter, and I. Mellman. 1998. Fc γ RII-B1 regulates the presentation of B cell receptor-bound antigens. *J. Immunol.* 161:2079.
48. Tsuyuki, S., J. Tsuyuki, K. Einsle, M. Kopf, and A. J. Coyle. 1997. Costimulation through B7-2 (CD86) is required for the induction of a lung mucosal T helper cell 2 (TH2) immune response and altered airway responsiveness. *J. Exp. Med.* 185:1671.
49. Krinzman, S. J., G. T. Desanctis, M. Cernadas, D. Mark, Y. S. Wang, J. Listman, L. Kobzik, C. Donovan, K. Nassr, I. Katona, D. C. Christiani, D. L. Perkins, and P. W. Finn. 1996. Inhibition of T cell costimulation abrogates airway hyper-responsiveness in a murine model. *J. Clin. Invest.* 98:2693.
50. Frew, A. J., and A. B. Kay. 1988. The relationship between CD4⁺ T lymphocytes, activated eosinophils and the magnitude of the allergen-induced late phase skin reaction in man. *J. Immunol.* 141:4158.
51. Corrigan, C. J., and A. B. Kay. 1992. T cells and eosinophils in the pathogenesis of asthma. *Immunol. Today* 13:501.
52. Harris, N., R. Peach, J. Naemura, P. S. Linsley, G. Legros, and F. Ronchese. 1997. CD80 costimulation is essential for the induction of airway eosinophilia. *J. Exp. Med.* 185:177.
53. Barata, L. T., S. Ying, Q. Meng, J. Barkans, K. Rajakulasingam, S. R. Durham, and A. B. Kay. 1998. IL-4- and IL-5-positive T lymphocytes, eosinophils, and mast cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *J. Allergy Clin. Immunol.* 101:222.
54. Keatings, V. M., B. J. O'Connor, L. G. Wright, D. P. Huston, C. J. Corrigan, and P. J. Barnes. 1997. Late response to allergen is associated with increased concentrations of tumor necrosis factor- α and IL-5 in induced sputum. *J. Allergy Clin. Immunol.* 99:693.
55. Bousquet, J., and F. Michel. 1992. Immunotherapy in rhinitis. In *Allergic and Non-Allergic Rhinitis: Clinical Aspects*. N. Mygind and R. Naclerio, eds. Munksgaard, Copenhagen, 136-148.
56. Wierenga, E. A., M. Snoek, J. D. Bos, H. M. Jansen, and M. L. Kapsenberg. 1990. Comparison of diversity and function of house dust mite-specific T lymphocyte clones from atopic and non-atopic donors. *Eur. J. Immunol.* 20:1519.
57. Jeannin, P., S. Lecoanet, Y. Delneste, J. Gauchat, and J. Y. Bonnefoy. 1998. IgE versus IgG4 production can be differentially regulated by IL-10. *J. Immunol.* 160:3555.
58. Yamashita, N., M. Takeno, S. Kaneko, Y. Mizushima, and T. Sakane. 1997. Therapeutic effects of α ferential induction of mite-specific T helper 0 clones. *Clin. Exp. Immunol.* 109:332.
59. Vrtala, S., T. Ball, S. Spitzauer, B. Pandjaitan, C. Suphioglu, B. Knox, W. R. Sperr, P. Valent, D. Kraft, and R. Valenta. 1998. Immunization with purified natural and recombinant allergens induces mouse IgG1 antibodies that recognize similar epitopes as human IgE and inhibit the human IgE-allergen interaction and allergen-induced basophil degranulation. *J. Immunol.* 160:6137.
60. Bonnefoy, J. Y., C. Plater-Zyberk, S. Lecoanet-Henchoz, J. F. Gauchat, J. P. Aubry, and P. Graber. 1996. A new role for CD23 in inflammation. *Immunol. Today* 17:418.