

Letter to the Editor

Effect of birch pollen-specific immunotherapy on birch pollen-related hazelnut allergy

To the Editor:

Birch pollen (BP) allergy is often associated with hazelnut allergy because of the homology between the major BP allergen Bet v 1 and the related hazelnut allergen Cor a 1.¹ Some studies have shown that BP-specific immunotherapy (BP-SIT) improves allergy to related foods,^{2,3} but others could not confirm this.^{4,5} Although a potential beneficial effect of BP-SIT on hazelnut allergy has been suggested, concrete data were not shown.⁶ Successful SIT might involve the induction of IgG and IgG₄ antibodies,⁷ which could block IgE binding. This may reduce IgE-mediated responses and inhibit IgE-facilitated allergen binding (IgE-FAB) and subsequent allergen presentation by B cells.⁸ In this double-blind, placebo-controlled study the effect of BP-SIT on hazelnut allergy was analyzed.

Nineteen adult patients with BP and hazelnut allergy (positive history and IgE measurement, skin prick test [SPT] response, or both for BP and hazelnut) were included in the study (4 male and 15 female patients; median, age 36 years; see Table E1 in this article's Online Repository at www.jacionline.org). Based on symptoms by history and during double-blind, placebo-controlled food challenge (DBPCFC), the hazelnut allergy was considered to be BP related. The study was approved by the ethics committee. All patients provided written informed consent. Exclusion criteria were pregnancy, unstable asthma, and oral corticosteroids or β -blocking agents. Patients were randomized, receiving either BP-SIT (n = 10; Alutard SQ; ALK-Abelló, Hørsholm, Denmark) or placebo (n = 9; histamine injections: 0.01, 0.1, 1, and 10 μ g/mL, ALK-Abelló), according to a modified cluster schedule. Both groups were comparable with respect to age, sex, BP- and hazelnut-specific IgE levels, and SPT responses (see Table E1).

Clinical outcome was analyzed by means of DBPCFC at inclusion and after 1 year of treatment (see Table E1, protocol in legend). The eliciting dose (ED) and symptom scores (visual analog scale for severity of oropharyngeal symptoms, data not shown) of the DBPCFC before treatment did not differ between the SIT and placebo groups, and both did not change significantly after 1 year of treatment in either group (shown for ED in Table E1).

Titration SPTs were performed with BP and hazelnut extract (ALK-Abelló) and the recombinant major allergens Bet v 1 (BP) and Cor a 1 (hazel pollen; Biomay, Vienna, Austria), as previously described.³ As control SPTs, grass pollen, house dust mite, and cat extract were tested (ALK-Abelló). SPT reactivity decreased significantly for BP, Bet v 1, and Cor a 1 only in the SIT group (Fig 1). SPT reactivity to hazelnut remained unchanged (Fig 1, A). SPT responses with control allergens also remained unchanged (not shown).

Specific IgE and IgG₄ levels to BP and hazelnut and the purified native major allergens Bet v 1 (BP) and Cor a 1 (hazelnut) were measured by means of RAST. Total IgG levels to hazelnut, Bet v 1, and Cor a 1 were analyzed by means of CAP or RIA.³ IgE levels to all allergens showed only minor fluctuations (see Fig E1 in this article's Online Repository at www.jacionline.org). IgG₄ antibodies against BP and Bet v 1 were significantly enhanced in the SIT group (4- to 5-fold at t = 6 months) but not in the placebo group. IgG₄ levels to hazelnut and Cor a 1 did not

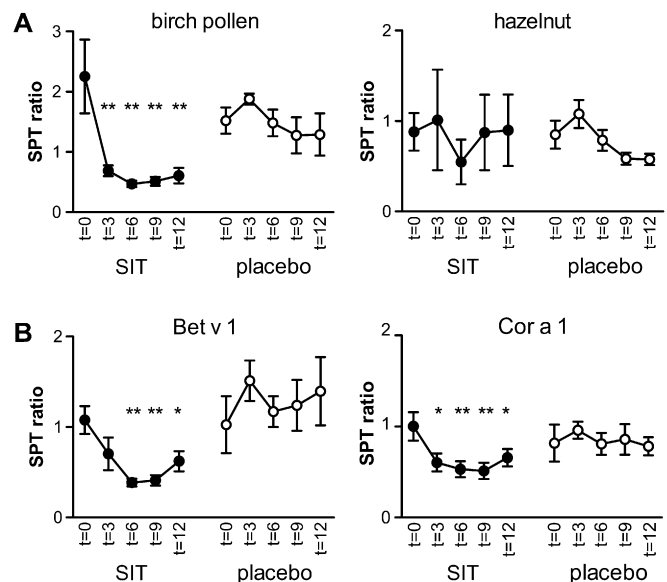


FIG 1. SPT reactivity to BP and hazelnut extract and the major allergens Bet v 1 and Cor a 1. SPTs were performed before and 3, 6, 9, and 12 months after the start of treatment. SPTs with recombinant Bet v 1 and Cor a 1 were titrated, and results of 2 μ g/mL were used for analysis. Means $\pm$ SEMs are shown for the SIT group (filled circles) and the placebo group (open circles). *P < .05 and **P < .01 compared with time 0.

change significantly. Total IgG levels to hazelnut, as well as to Bet v 1 and Cor a 1, were significantly increased in the SIT group (2- to 3-fold for hazelnut, 4- to 5-fold for Bet v 1, and 2- to 3-fold for Cor a 1 at 6 months; Fig 2 and see Fig E2 in this article's Online Repository at www.jacionline.org).

The effect of BP-SIT on IgE-FAB was analyzed by means of flow cytometry.⁸ IgE-allergen complexes were generated with BP extract (0.5 μ g/mL), hazelnut extract (10 μ g/mL), and recombinant Bet v 1 (0.1 μ g/mL) and Cor a 1 (0.1 μ g/mL). The binding of BP and Bet v 1 allergen-IgE complexes was inhibited in the SIT group by up to 80% after 12 months (Fig 2 and see Fig E2), which was significantly stronger than in the placebo group. Notably, by using hazelnut extract and Cor a 1, a similar significant inhibition of up to 70% was found in the SIT group compared with the placebo group, which is suggestive of cross-inhibition of IgE-FAB (Fig 2 and see Fig E2).

Together, these data show that BP-SIT did not result in clinical improvement of hazelnut allergy after 1 year of treatment. One explanation for the lack of clinical efficacy might be that BP-SIT only modulates the response to BP-related allergens but not to non-BP-related allergens in hazelnut and that these allergens were involved in the hazelnut allergy.⁹ However, the patients in our study showed hardly any IgE sensitization to non-BP-related allergens (CAP results for Cor a 8 and hazelnut extract depleted of Cor a 1 were low or negative, data not shown), strongly suggesting that the hazelnut allergy was BP-related. Therefore this is not a plausible explanation. Another reason could be that the dose of BP allergen in the SIT extract was too low or the treatment period was too short to induce clinical changes in hazelnut allergy.¹⁰ However, the same SIT preparation successfully reduced apple allergy in other studies, including our own.³ A third

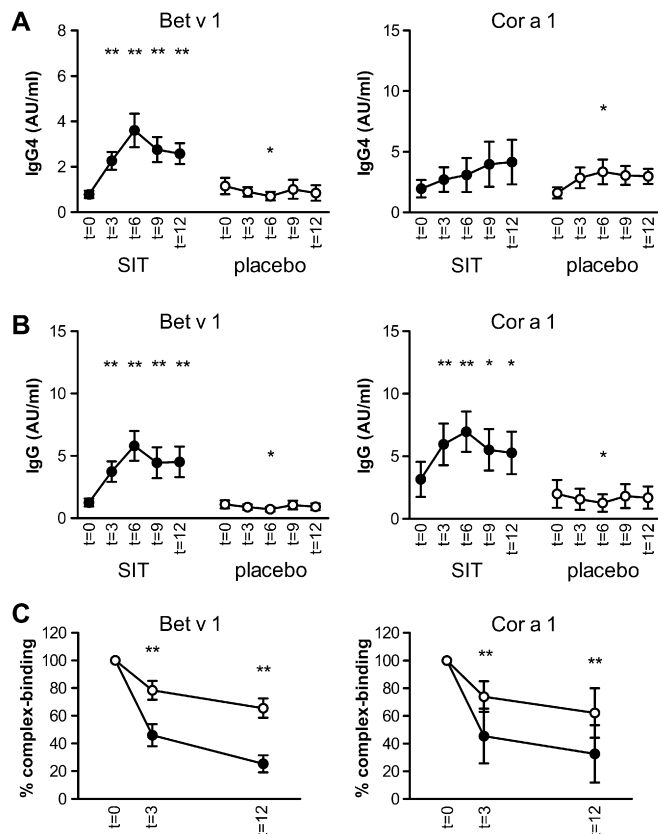


FIG 2. IgG₄ and IgG levels and IgE-FAB to the major allergens Bet v 1 and Cor a 1. **A** and **B**, IgG₄ levels (RAST; Fig 2, A) and IgG levels (RIA; Fig 2, B) were analyzed before and after treatment. **C**, IgE-antigen complexes bound to EBV-B cells were stained with anti-IgE antibodies. Mean fluorescence intensity (MFI) at 3 and 12 months was related to MFI at time 0 (set at 100%). Means $\pm$ SEMs are shown for the SIT group (filled circles) and the placebo group (open circles). * $P < .05$ and ** $P < .01$ compared with time 0 (Fig 2, A and B) or placebo (Fig 2, C).

explanation may be that it is hard to detect changes in the ED in patients with predominantly oropharyngeal symptoms. The studies that reported improvement of apple allergy by means of BP-SIT were not placebo-controlled.^{2,3} A placebo-controlled study was not able to detect changes.⁴ This shows that a properly controlled study setup is important in the analysis of the clinical effect of SIT on related food allergies.

Despite the lack of clinical efficacy, SPT responses were strongly reduced in the SIT group already after 3 months of treatment, which fits with previous data.³ This reduction was observed for BP, Bet v 1, and Cor a 1. Furthermore, SIT increased the levels of IgG₄ to BP and Bet v 1 and enhanced IgG to Bet v 1 and to both hazelnut and Cor a 1. A previous study with limited effect of sublingual BP-SIT on apple allergy, showed that IgG₄ levels to Bet v 1 were increased, whereas IgG₄ levels to Mal d 1 did not change.⁵ Sublingual SIT might be less potent than subcutaneous SIT, as is reflected in the difference in the increase in Bet v 1-specific IgG₄ levels between this study and our data. Unfortunately, in this previous study IgG levels to Mal d 1 were not analyzed, and a functional analysis of the induction of blocking antibodies was not performed. Our study shows that IgE-FAB was reduced by SIT to up to 80% after 12 months for BP, hazelnut, Bet v 1, and Cor a 1, suggesting that the increased IgG antibodies are functional blocking antibodies.

In conclusion, despite the lack of clinical efficacy, the data indicate that BP-SIT induces active immune modulation, leading to cross-reactive suppression of the Bet v 1-related hazelnut-specific response.

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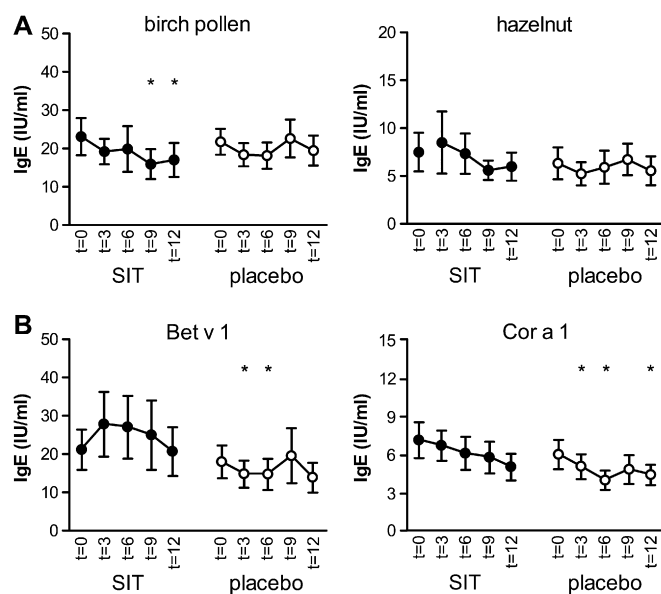


FIG E1. IgE levels to BP and hazelnut extract and the major allergens Bet v 1 and Cor a 1. IgE levels to BP and hazelnut extract (**A**) and native Bet v 1 and Cor a 1 (**B**) were measured by means of RAST in sera of patients before and treatment. Means $\pm$ SEMs are shown for the SIT group (*filled circles*) and the placebo group (*open circles*). * $P < .05$ compared with time 0.

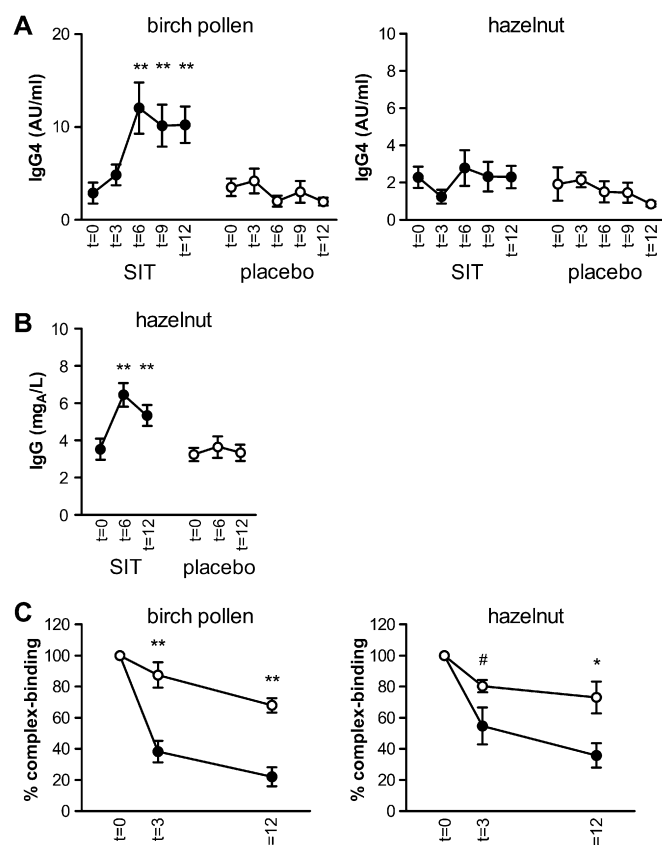


FIG E2. Ig G₄ and IgG levels and IgE-FAB to BP and hazelnut extract. **A** and **B**, IgG₄ levels, measured by means of RAST (Fig E2, A), and IgG levels, measured by means of CAP (Fig E2, B), to BP extract, hazelnut extract, or both were analyzed in sera of patients before and after treatment. **C**, For IgE-FAB, IgE-allergen complexes bound to EBV-B cells were stained with anti-IgE antibodies. Mean fluorescence intensity (MFI) at 3 and 12 months was related to MFI at time 0 (set at 100%). Means $\pm$ SEMs are shown for the SIT group (filled circles) and the placebo group (open circles). * $P < .05$, ** $P < .01$, and # $P = .068$ compared with time 0 (Fig E2, A and B) or placebo (Fig E2, C).

TABLE E1. Clinical characteristics of patients with BP and hazelnut allergy

Patient no.	Age (y)	Sex	Reported symptoms hazelnut allergy	History of AR or AA	IgE BP (kU _A /L)	IgE HN (kU _A /L)	SPT BP (ratio)*	SPT HN (ratio)*	HN ED (mg HN flour) t = 0	Most severe symptoms DBPCFC t = 0†	HN ED (mg HN flour) t = 12	Most severe symptoms DBPCFC t = 12‡
SIT group												
05	22	F	OA	AR	72.2	1.83	0.63	0.18	1,000	OA, dys	100	OA
06	56	F	OA	AR, AA	55.0	1.00	NA	NA	3,000	OA	1,000	OA
07	28	M	OA, nau, ap	AR, AA	>100	45.3	1.94	2.10	10	OA, nau, dys+FEV ₁ ↓	10	OA, nau
10	28	F	OA, rc, nau, vom	AR, AA	8.57	5.07	1.54	0.37	300	OA	300	OA
12	41	F	OA, dys, nau	AR, AA	85.2	3.58	1.88	0.32	1,000	OA	3,000	OA
14	62	F	OA	AR	83.4	2.57	0.55	0.81	10,000	OA	3,000	OA
15	20	M	OA, urt	AR	94.0	3.86	7.15	1.32	0.5	OA	1,000	OA
16	26	F	OA, rc, dys	AR	18.2	<0.35	1.67	0.66	0.1	OA	10	OA
18	38	F	OA	AR	>100	1.35	1.82	1.84	100	OA	10	OA
19	46	F	OA	AR, AA	24.6	1.10	1.52	0.69	3,000	OA	10,000	OA
Median	33				77.8	2.20	1.67	0.69	650		650§	
Placebo group												
01	41	F	OA, nau	AR, AA	14.3	<0.35	1.85	0.53	0.1	OA, dys	10	OA, dys
02	25	F	OA, rc	AR	91.2	2.39	1.62	0.88	0.01	OA	10	OA
03	19	F	OA, rc, dys, urt	AR, AA	>100	4.15	1.24	0.65	0.1	OA	1,000	OA
04	32	F	OA	AR	66.8	0.62	2.97	1.87	1,000	OA	1,000	OA
08	18	F	OA, ap, dia	AR, AA	>100	1.62	0.73	0.25	1,000	OA, nau	3,000	OA, nau
09	38	F	OA	AR	45.5	3.73	1.14	0.56	3,000	OA	1,000	OA
11	43	M	OA, rc, dys	AR, AA	47.2	0.87	0.98	0.94	100	OA	3,000	OA, cou, dys+FEV ₁ ↓
13	55	M	OA, dys	AR, AA	11.3	<0.35	1.58	1.17	300	OA	0.01	OA
17	36	F	OA, rc, dys	AR, AA	82.7	2.67	1.56	0.81	100	OA	1,000	OA
Median	36				66.8‡	1.62‡	1.56‡	0.81‡	100‡		1,000‡§	

AA, allergic asthma; *ap*, abdominal pain; *AR*, allergic rhinitis; *cou*, cough; *dia*, diarrhea; *dys*, dyspnea; *dys+FEV₁↓*, dyspnea with decrease in FEV₁; *ED*, eliciting dose during DBPCFC; *HN*, hazelnut; *NA*, not available (patient exhibited hyperreactivity in SPTs, data were excluded from analysis); *nau*, nausea; *OA*, oral allergy symptoms; *rc*, rhinoconjunctivitis; *urt*, urticaria; *vom*, vomiting.

*SPT ratio: allergen-specific wheal reaction in millimeters squared divided by wheal reaction of positive control (histamine). SPT ratios were regarded as positive when 0.25 or greater.

†DBPCFC recipes contained defatted hazelnut flour, 0.01 mg, 0.1 mg, 0.5 mg, 1 mg, 10 mg, 100 mg, 300 mg, 1 g, and 3 g (15.5% protein, 3 g corresponds to 3 hazelnuts; FARRP, University of Nebraska, Lincoln, Neb), masked in wheat instant cereal–cinnamon mix and apple sauce. Four placebo doses were randomly interspersed. A negative DBPCFC result was followed by an open provocation with 1, 3, and 10 g of raw hazelnuts. The challenges were conducted in a hospital setting, with careful monitoring of the patients. Full emergency treatment was readily available. The challenge was discontinued when objective symptoms occurred, when convincing subjective symptoms occurred at least 3 times, or when the highest dose was reached. The ED was determined as the lowest dose inducing convincing (subjective) symptoms.

‡SIT group versus placebo group: not significant.

§Before versus after treatment: not significant (Student *t* test).