

Letter to the Editor

Vaccination with genetically modified birch pollen allergens: Immune and clinical effects on oral allergy syndrome

To the Editor:

Birch pollen-associated oral allergy syndrome (OAS) is mainly caused by IgE antibodies that cross-react with the major birch pollen allergen, Bet v 1, and with the major allergens from apple (Mal d 1), celery (Api g 1), and carrot (Dau c 1), as well as other plant-food allergens.¹⁻³ Preclinical data had indicated that vaccination with genetically engineered hypoallergenic derivatives of Bet v 1 (Bet v 1 fragments, Bet v 1 trimer) induced IgG antibodies that cross-react with Bet v 1-related plant-food allergens.^{4,5}

We have recently performed a preseasonal immunotherapy trial with the genetically engineered hypoallergenic derivatives of Bet v 1.⁶ Vaccination induced high levels of IgG antibodies that inhibited IgE recognition of Bet v 1, Bet v 1-induced release of inflammatory mediators, and Bet v 1-induced boosts of IgE production.⁶ In this substudy, we analyzed the effects of vaccination with genetically modified Bet v 1 derivatives on immune responses to food allergens in patients with birch pollen-associated OAS.

The subcutaneous injection immunotherapy study was conducted as a multicenter, placebo-controlled, double-blind randomized trial over a period of 12 months.⁶ Patients received 1 preseasonal treatment course with an equimolar mixture of Bet v 1 fragments,⁴ a hypoallergenic Bet v 1-

trimer,⁵ or aluminium hydroxide alone (placebo). The study was approved by the local ethical committees, and written informed consent was obtained from each patient.

We analyzed 44 patients from 1 study center (Vienna; placebo: n = 19, fragments: n = 12, trimer: n = 13) who had allergic symptoms upon ingestion of birch pollen-associated plant food (apples: n = 42, hazelnuts: n = 36, carrot or celery: n = 21, other plant-derived foods: n = 16) and who had eaten the offending foods during the study period and were thus able to state whether their symptoms had or had not improved. Patients had on an average received 8.18 injections (placebo: 8.42, range 5-11; treatment: 8.00, range, 3-10). Actively treated patients had received a cumulative injected dose of 150.0 µg recombinant protein on average (range, 4-245 µg). Twenty-five of the patients were also sensitized to other pollen (grass pollen: n = 23, weed pollen: n = 14). IgE immunoblot experiments had revealed that in 43 of the patients, birch pollen allergy was caused exclusively by Bet v 1 and only 1 patient (patient F6) also had IgE antibodies to Bet v 2.

The presence of allergen-specific IgE, IgG₁₋₄, and IgA antibody levels was analyzed in serum samples obtained before and after treatment by enzyme-linked immunosorbent assay.⁷ The Spearman rank correlation test was used to assess correlations between changes of antibody levels. Active treatment induced a significant rise of IgG₁ antibodies reactive with Mal d 1 (fragments: +130%, $P < .01$; trimer: +100%, $P < .01$), Api g 1 (fragments: +46%, P not significant; trimer: +43%, $P < .05$) (Fig 1)

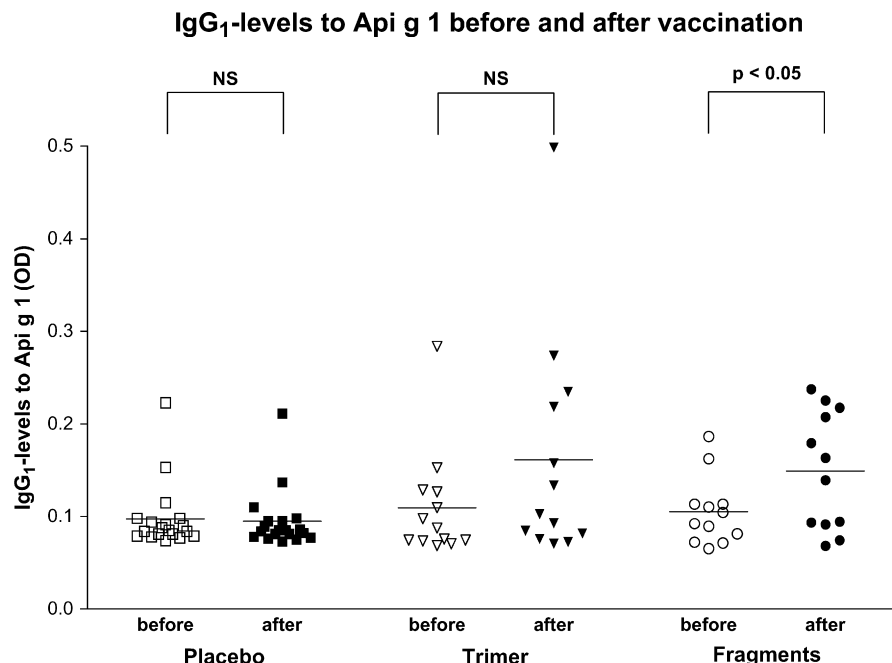


FIG 1. IgG₁ levels to Api g 1 were measured by enzyme linked immunosorbent assay in sera obtained before and after treatment. Optical densities (OD) corresponding to the levels of specific IgG₁ are shown on the y-axis. Horizontal bars represent mean IgG₁ levels. NS, Not significant.

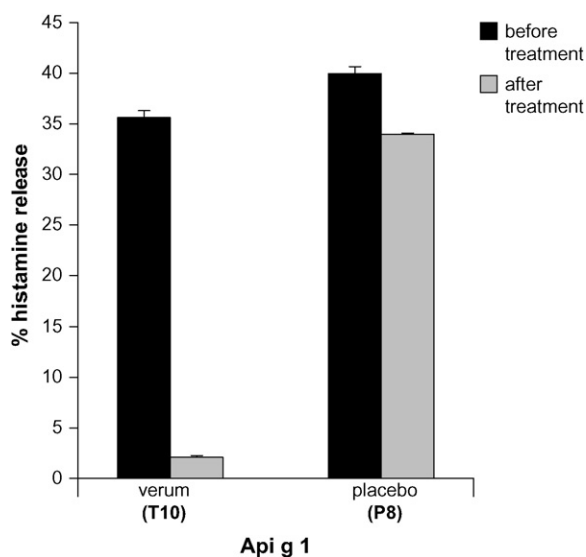


FIG 2. Inhibition of Api g 1-induced histamine release by treatment-induced antibodies. Percentages of histamine release (y-axis) induced with 0.1 μ g/mL rApi g 1 in the presence of sera from a trimer-treated (T10) patient (*left panel*) before and after (ie, containing Api g 1-specific IgG) treatment and in the presence of sera from a placebo-treated (P8) patient (*right panel*) before and after treatment.

and Dau c 1 (fragments: +76%, $P < .01$; trimer: +33%, $P < .01$) as compared to placebo treatment (Mal d 1: -3%, Fig 1; Api g 1: -2%; Dau c 1: -2%, all P values not significant). Rises of IgG₁ antibodies recognizing Mal d 1, Api g 1, and Dau c 1 were significantly correlated to rises of IgG₁ antibody levels to Bet v 1 during treatment ($P < .01$) and to the cumulative injected dose of active preparations which the patients received ($P < .01$). We also found increases of IgG₄ antibodies to Mal d 1 (fragments: +91%, $P =$ not significant; trimer: +92%, $P = .02$) and Dau c 1 (fragments: +33%, $P < .05$; trimer: +24%, $P =$ not significant) but these were less pronounced than those observed for IgG₁ responses. We did not find significant changes of IgA, IgE, IgG₂, and IgG₃ antibodies to Mal d 1, Dau c 1, and Api g 1.

Whether sera containing therapy-induced IgG antibodies can inhibit food allergen-induced basophil degranulation was studied by histamine release experiments as described.⁶ Posttherapy serum from a patient who had received treatment with trimer inhibited Api g 1-induced basophil degranulation by 94.1% as compared to serum obtained before therapy (Fig 2). A similar though less pronounced effect was observed for Mal d 1-induced histamine release: posttreatment serum from a fragment-treated patient inhibited Mal d 1-induced histamine release to an extent of 28% (not shown). When pre- and posttherapy sera from placebo-treated patients were used for similar experiments, no relevant inhibition of histamine release was observed.

For the assessment of OAS, patients completed questionnaires before therapy and after the first birch

pollen season following therapy. Patients were asked how often (regularly/rarely/never) they suffered from symptoms of OAS (oral itching/swelling of lips or tongue/other symptoms) upon ingestion of certain foods (apples/hazelnuts/carrots or celery/other foods) and how frequently they ate these foods. In the second questionnaire, patients were asked whether their symptoms had changed (worse/unchanged/improved/completely resolved) following therapy.

Despite the short course of treatment, we found that 7 of 25 actively treated patients (fragments: 5; trimer: 2) but only 1 of the 19 placebo-treated patients reported improvement of OAS. Two actively treated and 2 placebo-treated patients reported a deterioration of OAS. Both of these actively treated patients had received low doses of the preparations (patient F6: 28 μ g; patient F11: 4 μ g), and 1 of these patients (patient F6) was sensitized to Bet v 1 as well as Bet v 2. No change of OAS was reported in 16 actively treated and 16 placebo-treated patients.

We believe that the extensive cross-reactivity between the major allergen of birch, Bet v 1, and related plant-food allergens represents the immunologic basis for the beneficial effects of vaccination with genetically modified Bet v 1 on birch pollen-associated OAS. Therapy-induced Bet v 1-specific IgG corecognizing the cross-reactive food allergens may inhibit IgE binding via the following mechanisms: as the Bet v 1 trimer resembles some aspects of the fold of wild-type Bet v 1, it is possible that trimer-induced IgG may recognize the very same epitopes as those that are recognized on the Bet v 1 wild type. The Bet v 1 fragments are unfolded molecules and hence will induce IgG antibodies that either bind in the vicinity of IgE epitopes or recognize parts of conformational IgE epitopes and therefore inhibit IgE binding to the natural allergen by steric hindrance or occupation of binding sites. Finally, it is possible that certain IgG antibodies may, on binding to the natural allergen, induce a conformational change affecting IgE epitopes.

Our results may be considered encouraging for several reasons. First, the injection of purified single-antigen derivatives induced cross-reactive antibody responses against the related food allergens and signs of a beneficial effect on OAS were achieved after 1 short treatment course. Second, it is possible that a longer treatment period as is performed in the course of routine immunotherapy or a mucosal application of treatment may improve the results. Furthermore, the beneficial effect of this treatment on symptoms of oral allergy may have been more pronounced had the patients completed the questionnaires later in the year instead of shortly after the birch pollen season.

In summary, we believe that the current data should encourage further larger clinical studies of the use of genetically engineered pollen allergens for concomitant immunotherapy treatment of OAS that are objectively monitored by double-blind, placebo-controlled challenge with purified recombinant food allergens rather than with natural food extracts.⁸

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