Birch pollen immunotherapy results in long-term loss of Bet v 1–specific T_H2 responses, transient T_R1 activation, and synthesis of IgE-blocking antibodies

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Background: Early events of specific immunotherapy (SIT) are induction of allergen-specific IL-10–producing T_R1 cells and production of IgG antibodies, but there is little knowledge about the long-term immune mechanisms responsible for sustained allergen tolerance.

Objective: Bet v 1–specific immune responses of 16 patients with birch pollen allergy were characterized up to 54 months at defined time points before, during, and after a 3-year period of SIT.

Methods: We sought to analyze allergen-specific T- and B-cell responses. Bet v 1–specific IL-5–, IFN-γ–, and IL-10–secreting T cells were quantified in peripheral blood, and birch pollen–specific IgE and IgG antibody levels were determined in serum. Furthermore, the inhibitory capacity of SIT-induced IgG was evaluated by blocking allergen binding to IgE and inhibition of facilitated allergen presentation.

Results: Seasonal increases in Bet v 1–specific T_H2 cell numbers ceased to appear after the first year of SIT without deviation to a T_H1-dominated immune response. Furthermore, the frequency of IL-10–producing T_R1 cells, which had increased during the first year of SIT, returned to pretreatment levels in the second year. In contrast, allergen-specific IgG antibody concentrations continuously increased during SIT but started to decrease after cessation of treatment. Functional analysis confirmed the ability of the IgG antibodies to inhibit IgE-allergen interactions, which peaked at the end of SIT but then slowly started to decrease.

Conclusion: Long-term allergen tolerance achieved by SIT is associated with the development of peripheral T-cell tolerance characterized by decreased reactivity of Bet v 1–specific T_H2 cells and enriched allergen-specific IgG competing with IgE antibodies for allergen binding. (J Allergy Clin Immunol 2012;130:1108-16.)

Key words: *Respiratory allergy, specific immunotherapy, allergen tolerance, Bet v 1, IgE, IgG, blocking antibodies, regulatory T cells*

The long-lasting clinical efficacy of specific immunotherapy (SIT) has been well documented,[1-5] but the underlying immune mechanisms are still a focus of intensive research. Thus far, most immunologic studies have concentrated on the development of allergen tolerance during the first 1 to 2 years of SIT. Initially, a shift from an allergen-specific T_H2 to a T_H1 immune response, resulting in the correction of an imbalance between T_H2 and T_H1 cells in allergic subjects, was regarded as an important mechanism of successful SIT.[6-8] Later, it appeared that another T-cell subset called regulatory T (Treg) cells plays a major role in the development of allergen tolerance.[9] We and others could show that IL-10–producing type 1 Treg (T_R1) cells, which exhibit distinct features discriminating them from $CD4^+CD25^+CD127^{low/-}$ forkhead box protein 3 $(Foxp3)^+$ Treg cells,[10-14] are induced during the first few months of SIT.[13,15,16] In addition, an increased production of allergen-specific IgG, especially IgG_4, antibodies was noticed early on during SIT.[15,17-19] Because these antibodies were able to block allergen-IgE interactions *in vitro*, a functional role in inhibiting IgE-mediated allergic reactions was suggested.[20]

Although these observations helped to elucidate mechanisms of how SIT promotes the induction of allergen tolerance, data about immunologic alterations associated with long-term efficacy of SIT, in particular covering the time after its discontinuation, are very sparse. Of note, to our knowledge, there are no reports of longitudinal studies about the potential role of allergen-specific T-cell subsets in sustaining SIT-induced allergen tolerance beyond the treatment period. Only a few studies thoroughly analyzed humoral immune responses by following allergen-specific antibody concentrations in patients who completed either subcutaneous or sublingual SIT with aeroallergens.[21-24] Although all showed increased IgG_4 serum concentrations, correlation with the clinical outcome was inconsistent, raising the question of whether other immunologic markers, such as the functional ability of these antibodies to block allergen-IgE interaction, are better suited to assess the efficacy of SIT[20,22] and pointing to the need for clinical studies more closely investigating the immune alterations underlying the long-term outcome of allergic patients treated by means of SIT.

METHODS

Patients

The investigated cohort consisted of 16 patients with birch pollen allergy. Inclusion/exclusion criteria and detailed SIT-induced alterations during the first year of therapy of all but 1 patient were previously published.[13] Briefly, participants had a history of moderate-to-severe birch

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Abbreviations used
FAP: Facilitated allergen presentation
Foxp3: Forkhead box protein 3
PMP: Paramagnetic particle
SIT: Specific immunotherapy
SPT: Skin prick test
Treg: Regulatory T

pollen–induced rhinoconjunctivitis and/or mild-to-moderate asthma, positive skin prick test (SPT) and nasal provocation responses to birch pollen extract (ALK Prick SQ and ALK-depot SQ, respectively; both from ALK-Abelló, Hørsholm, Denmark), and specific serum IgE reactivity against birch pollen extract and rBet v 1 (Phadia ImmunoCAP System; Phadia, Uppsala, Sweden). Patients with a history of clinically relevant allergies against other pollens, polysensitization with perennial symptoms, or chronic nonallergic asthma were excluded. Withdrawal of subjects occurred because of non–treatment-related events. The study was approved by the Ethics Committee of the Medical Faculty of Philipps University, Marburg, Germany; all patients provided written informed consent to participate in the trial.

Study design

Details of the SIT protocol have been reported previously.[13] In brief, patients received weekly up-dosing injections subcutaneously until the maintenance dose of 100,000 standard quality units of birch pollen allergen per injection was reached, followed by monthly maintenance injections for 3 years.

Further details on evaluation of birch pollen extract SPT reactivity and assessment of SIT-treated patients' symptoms during natural birch pollen exposure are provided in the Methods section in this article's Online Repository at www.jacionline.org.

Blood samples and analysis of PBMCs

Preparation of peripheral blood samples and subsequent characterization of PBMCs by means of ELISpot and ELISA analysis are described in detail in the Methods section in this article's Online Repository.

Analysis of allergen-specific serum immunoglobulins

Levels of total IgE, specific IgE, IgG and IgG_4 antibodies against *Betula verrucosa* (birch) pollen allergen, and specific IgE antibodies against rBet v 1 were measured by using the Phadia ImmunoCAP System, according to the manufacturer's instructions. In addition, specific IgG_1 and IgG_4 antibodies against birch pollen extract and nBet v 1 and rBet v 1 were determined by means of Western blotting (see additional information in the Methods section in this article's Online Repository).

For further details on facilitated allergen presentation (FAP), IgE blocking factor assay, and depletion of IgG antibodies, see the Methods section in this article's Online Repository.

Statistical analysis

Comparison of paired samples with at least 6 patients before and at different time points during/after SIT was done by using the nonparametric Wilcoxon signed-rank test with SPSS 17.0 software (SPSS, Chicago, Ill). Differences were considered statistically significant at P values of less than .05, less than .01, and less than .001.

RESULTS

Sustained clinical efficacy of birch pollen SIT

Clinical efficacy of SIT was assessed by reporting the extent of allergic symptoms during natural birch pollen exposure and determining the SPT reactivity to birch pollen extract. Amelioration of allergic symptoms, which was already achieved during the first SIT year,[13] persisted over the entire treatment period and the 2 years thereafter (Table I). In addition, SPT reactivity was significantly decreased during SIT but returned to pretreatment values 2 years after stopping therapy in the follow-up subgroup (Table I).

Long-term loss of Bet v 1–specific T_H2 cell responses

Compared with the first SIT year, when numbers of Bet v 1–specific IL-5–secreting T cells still increased because of natural allergen exposure (month 6), a markedly reduced allergen-specific T_H2 cell response was noticed during the birch pollen seasons in the second and third SIT years (months 18 and 30) and in the subsequent 2 years after termination of SIT (m42 and m54; Fig 1). Although Bet v 1–specific IFN-γ–producing T-cell numbers also significantly decreased during active treatment (Fig 1), their frequency increased again after completion of SIT, reflecting the condition seen in healthy control subjects during the birch pollen season.[13]

Transient induction of Bet v 1–specific T_R1 cells

As previously reported, Bet v 1–specific T cells, characterized as T_R1 cells by suppression of T effector cell proliferation in an IL-10–dependent manner and the lack of Foxp3 expression, were induced 3 months after SIT initiation, and their numbers continued to increase until the first birch pollen season (month 6; Fig 1).[13] However, allergen-specific T_R1 cell activation returned to pretreatment levels despite ongoing birch pollen immunotherapy for another 2 years. Thus clinical improvement was sustained in SIT-treated patients despite an only temporary activation of Bet v 1–specific T_R1 cells.

Prolonged decrease of IL-5 and IL-10 secretion by PBMCs

Although increased frequencies of the above-described Bet v 1–specific T-cell subsets were not detected by using ELISpot analysis after the first SIT year, we determined the secretion of the respective cytokines (IL-5, IFN-γ, and IL-10) by analyzing the supernatants of Bet v 1–stimulated PBMCs. Compared with pretreatment levels, IL-5 and IL-10 concentrations were enhanced in the first birch pollen season (month 6; see Fig E1 in this article's Online Repository at www.jacionline.org) but then continuously decreased until the end of the follow-up period. In addition, IFN-γ levels were increased during neither the SIT nor the post-SIT periods (see Fig E1, *B*). Thus the cytokine ELISA profile of PBMCs from SIT-treated patients confirmed the loss of a Bet v 1–specific T_H2 cell response after the first year of SIT, the absence of a T_H1 cell shift, and the transient activation of Bet v 1–specific T_R1 cells, respectively.

Alterations of birch pollen–specific immunoglobulin concentrations

Both birch pollen–specific and Bet v 1–specific IgE levels did not change significantly during SIT or in the subsequent 2 years thereafter (see Fig 2, *A*, and Fig E2 in this article's Online