

Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis

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Background: Recombinant DNA technology has the potential to produce allergen-specific immunotherapy vaccines with defined composition.

Objective: To evaluate the effectiveness of a new recombinant birch pollen allergen vaccine in patients with birch pollen allergy.

Methods: A multicenter, randomized, double-blind, placebo-controlled trial was undertaken to compare the following 3 vaccines in 134 adults with birch pollen allergy: recombinant birch pollen allergen vaccine (rBet v 1a), licensed birch pollen extract, natural purified birch pollen allergen (nBet v 1), and placebo. Patients received 12 weekly injections followed by monthly injections of the maintenance dose containing 15 µg Bet v 1 for 2 years.

Results: Significant reductions (about 50%) in rhinoconjunctivitis symptoms (rBet v 1, $P = .0002$; nBet v 1, $P = .0006$; birch extract, $P = .0024$), rescue medication (rBet v 1, $P = .0011$; nBet v 1, $P = .0025$; birch extract, $P = .0063$), and skin sensitivities ($P < .0001$) were observed in the 3 actively treated groups compared with placebo during 2 consecutive pollen seasons. Clinical improvement was accompanied by marked increases in Bet v 1-specific IgG levels, which were higher in the rBet v 1-treated group than in the birch and nBet v 1-treated groups. New IgE specificities were induced in 3 of 29 patients treated with birch pollen extract, but in none of the 32 rBet v 1-treated or 29 nBet v 1-treated patients. No severe systemic adverse events were observed in the rBet v 1-treated group. **Conclusion:** The rBet v 1-based vaccine was safe and effective in treating birch pollen allergy, and induced a highly specific immune response. (*J Allergy Clin Immunol* 2008;122:951-60.)

Key words: Recombinant allergens, immunotherapy, Bet v 1, allergic rhinitis, IgE, IgG4, allergy, allergen

Since the introduction of immunotherapy, the only treatment option for respiratory IgE-mediated allergy has been the administration of natural allergen extracts.¹ However, with natural

Abbreviations used

nBet v 1: Purified natural Bet v 1

rBet v 1: Recombinant Bet v 1

SAE: Serious adverse event

extracts the allergen content may vary between batches,^{2,3} and consequently the clinical effects may also vary considerably.⁴ In addition, natural extracts contain a variety of substances that promote the development of an allergic immune response.^{5,6} Injection immunotherapy with birch pollen extract has been reported to induce additional sensitization.⁷ Whether these iatrogenic sensitizations are clinically relevant is unknown, but it has been shown that mite immunotherapy can generate anaphylactic reactions to snails.⁸ Furthermore, reports indicate that allergen extracts can be contaminated with allergens from foreign sources⁹ and with endotoxins, which may have an unpredictable influence on the immune response.¹⁰

One method of improving allergen-specific immunotherapy is to use recombinant allergens produced by DNA technology, which allows highly specific treatment of patients according to their sensitization profile.¹¹ The current study examined whether immunotherapy with a recombinant construct of the major birch (*Betula verrucosa*) pollen allergen (Bet v 1) could be effective for the treatment of birch pollen allergy. Because its biological activity has been demonstrated in patients,^{12,13} recombinant Bet v 1 (rBet v 1) was compared in a multicenter, double-blind, placebo-controlled clinical trial with natural birch pollen extract, purified natural birch pollen extract (nBet v 1), and placebo.

METHODS

Patients

Patients included in the study were 18 to 50 years of age, with at least a 2-year history of birch pollen-related rhinoconjunctivitis (with or

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served as an expert witness for biostatistics. L. K. Poulsen has received research support from Siemens and has served as an expert witness for Novozymes. The rest of the authors have declared that they have no conflict of interest.

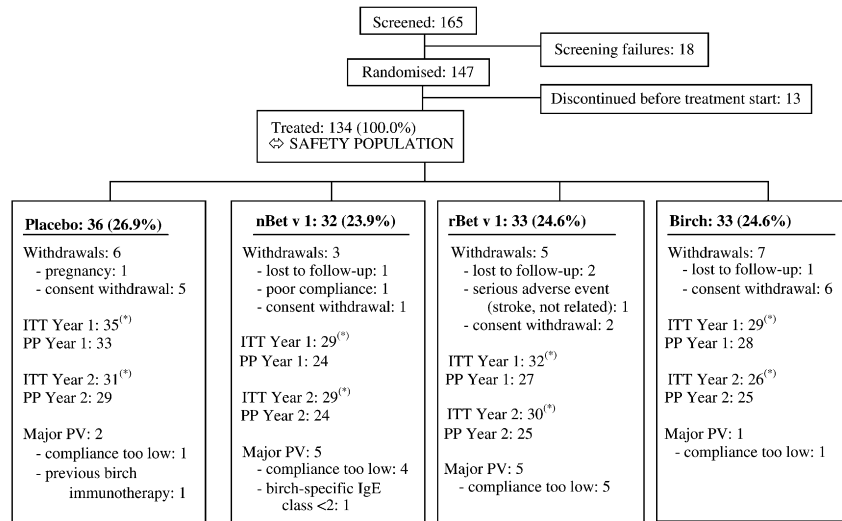
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^(*) Patients providing a diary card during the pollen season

PV: Protocol violation

ITT: included in the Intent to Treat population during the corresponding treatment year

PP: included in the Per Protocol population (no major PV) during the corresponding treatment year

FIG 1. Distribution of patients.

TABLE I. Demographic characteristics of patients

Characteristics of patients	Placebo (N = 35)	nBet v 1 (N = 29)	rBet v 1 (N = 32)	Birch pollen (N = 29)
Sex				
Male, no. (%)	19 (54.3%)	18 (62.1%)	17 (53.1%)	19 (65.5%)
Female, no. (%)	16 (45.7%)	11 (37.9%)	15 (46.9%)	10 (34.5%)
Age (y)				
Mean (± SD)	33.0 ± 9.5	34.1 ± 8.9	32.2 ± 8.6	34.1 ± 8.8
Range	19.2-50.9	20.6-48.8	19.3-49.6	21.1-50.8
No. of years with birch pollen allergy ± SD	13.9 ± 9.7	12.5 ± 8.1	12.3 ± 7.3	14.0 ± 7.8
Birch specific IgE				
Mean (IU/mL)	29.9	22.6	27.3	25.5
Range	1.1-96.1	0.4-108.0	1.4-96.5	1.3-100.0
Skin prick test				
mean wheal diameter (mm)	6.4	6.6	6.8	6.2
Range	4.1-10.2	3.0-13.0	3.0-16.3	3.0-11.1
Coexisting conditions, no. (%)				
Rhinitis	35 (100%)	29 (100%)	32 (100%)	29 (100%)
Conjunctivitis	35 (100%)	28 (96.6%)	32 (100%)	29 (100%)
Asthma	8 (22.9%)	5 (17.2%)	5 (15.6%)	8 (27.6%)
Eczema	5 (14.3%)	3 (10.3%)	4 (12.5%)	8 (27.6%)

without Global Initiative for Asthma grade 1-2 asthma)¹⁴; a positive skin prick test (wheal diameter >3 mm) in response to a licensed birch pollen extract, rBet v 1, and nBet v 1 (Stallergènes SA, Antony, France); and serum-specific IgE levels ≥class II (CAP System; Phadia, Uppsala, Sweden) to birch allergen. Patients with perennial rhinitis, uncontrolled asthma, previous immunotherapy, and the usual contraindications to immunotherapy were excluded from the study.¹⁵ Exclusion of patients sensitized and clinically reactive to other allergens (5-grass, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, dog, cat, horse, *Alternaria*, and *Cladosporium*) present during the birch pollen season avoided methodological bias.

The study was performed in accordance with the Declaration of Helsinki, local Ethics Committees, and Good Clinical Practice guidelines.

Study design

The study was performed in 5 European centers (Copenhagen, Gothenburg, Milan, Strasbourg, and Vienna) as a double-blind, placebo-controlled, parallel-group trial. A baseline period in 2003 was followed by randomization by the minimization method, considering symptom severity and degree of birch sensitization. One-hundred forty-seven patients were randomized to 4 parallel groups receiving subcutaneous injections of either birch pollen extract, rBet v 1, nBet v 1, or placebo. The different study sites were equally represented in the 4 groups. The treatment period was from October 2003 to October 2005. The mean total score for rhinoconjunctivitis during the 2003 baseline pollen peak was 8.15 (±SD 4.75) in the placebo group, 6.85 (±SD 3.57) in nBet v 1 group, 8.14 (±SD 5.62) in the rBet v 1 group, and 8.10 (±SD 4.00) in the birch pollen group. The reduced baseline symptom score (~20%) in the nBet v 1 group was

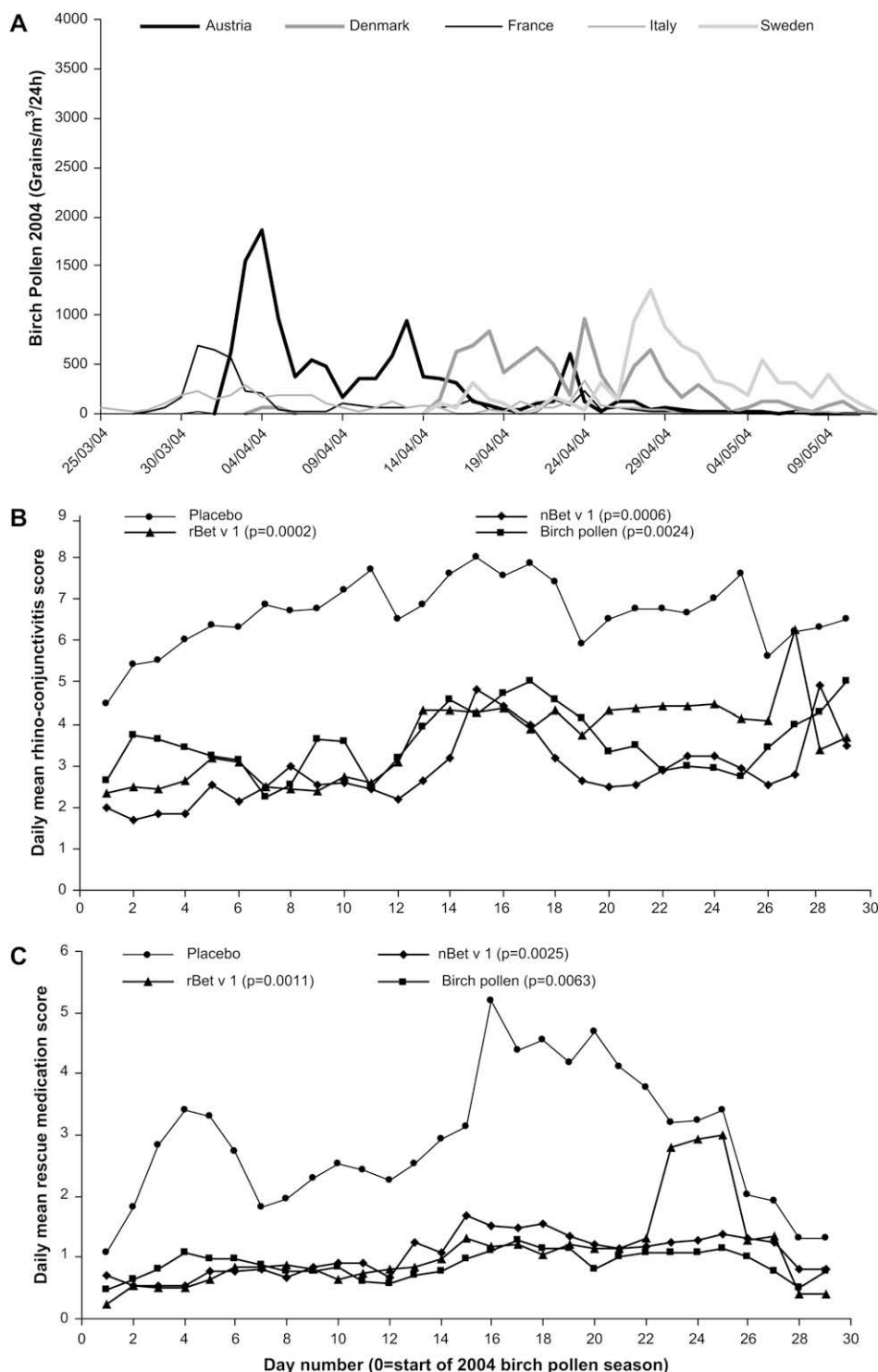


FIG 2. Daily birch pollen counts (grains/m³) and mean rhinoconjunctivitis scores. **A-C**, 2004 Season; **D-F**, 2005 season. **A, D**, Birch pollen counts on given dates; **B, E**, daily mean rhinoconjunctivitis scores; **C, F**, daily mean rescue medication scores for each treatment group. *P*, Placebo vs each active treatment group.

probably caused by the fact that 13 patients decided to not participate in the study between randomization and the start of the clinical trial. Thus, the build-up phase started 6 months before the pollen season, and all patients received maintenance treatment for at least 7 weeks before the pollen peak.

During the build-up phase, patients received once-weekly increasing doses of allergen or placebo. Aluminum hydroxide-adsorbed vaccines (a classic galenic

form in Europe) with Bet v 1 concentrations of 0.5 µg/mL, 5 µg/mL, and 50 µg/mL were used. Bet v 1 quantification was performed by ELISA. The amount of bound allergens was verified by the fact that the allergen concentrations were known before formulation. Vaccination was started with 0.1 mL of the lowest concentration. At each injection, the dose was doubled until 0.4 mL of the 5-µg/mL preparation was reached. Injections were continued with 0.6 mL and 0.8 mL

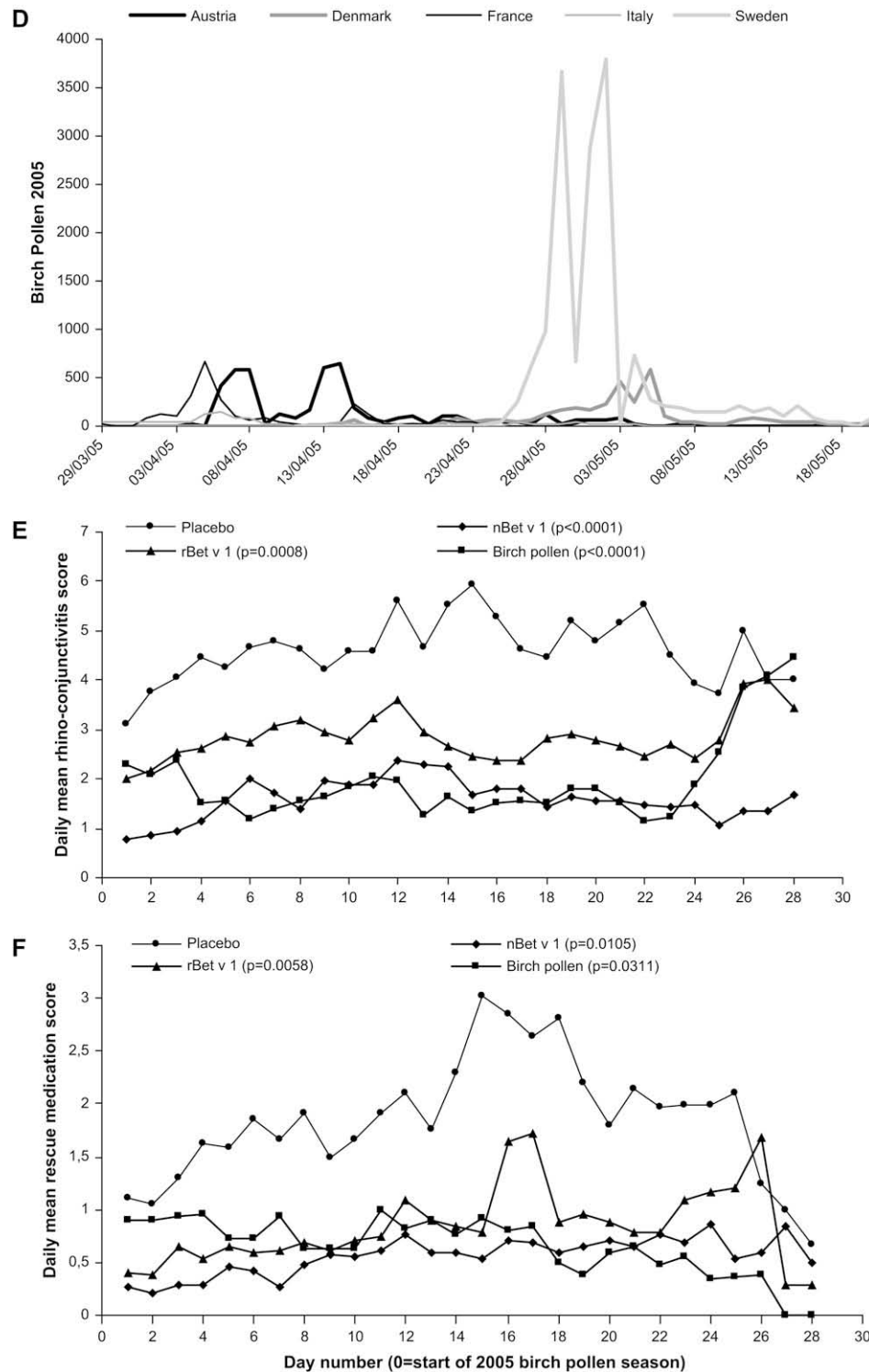


FIG 2. (Continued).

of the 5- μ g/mL preparation and then with 0.1 mL, 0.2 mL, and 0.3 mL of the 50- μ g/mL preparation or the maximum tolerated dose. The 3 active products and the placebo preparation were completely identical because of their similar content of aluminum hydroxide gel. To maintain blinding with regard to skin irritation, the placebo preparation contained 0.0005 mg/mL, 0.005 mg/mL, and 0.05 mg/mL histamine dihydrochloride.

After the build-up phase, patients received monthly injections of 15 μ g Bet v 1 or placebo for 2 years. If the patient was symptomatic on the day of injection, had experienced systemic reactions, or had mounted a considerable (>8 cm) local, late-phase reaction after the previous injection, the dose was reduced. After each injection, patients remained in hospital for 30 minutes.

TABLE II. Comparison of daily average total score for rhinoconjunctivitis between treatment groups; adjusted mean differences vs placebo

	Adjusted mean	95% CI†	Adjusted <i>P</i> value‡
nBet v 1 (n = 32)	3.85	1.55-6.16	.0006*
rBet v 1 (n = 33)	3.32	1.07-5.57	.0002*
Birch	3.41	1.10-5.73	.0024

ANOVA on the ranks provides *P* values.

*Significant at 5% level (2-sided test).

†ANOVA on the efficacy variable with treatment group and center as factors.

‡Bonferroni adjustment against multiplicity.

Skin prick tests using glycerol-saline solution of nBet v 1, rBet v 1, and birch extract were performed in quadruplicate at enrollment before immunotherapy, and after 1 and 2 years of treatment. Wheal responses were recorded after 15 minutes by computerized digital planimetry. Serum samples were taken before immunotherapy, after the first treatment season, and after 1 and 2 years of treatment. Serum samples were stored at -20°C to assess allergen-specific antibody levels.

Study materials

The recombinant Bet v 1a isoform^{16,17} was expressed in *Escherichia coli*. nBet v 1 was purified from defatted birch pollen (*B verrucosa*) as described previously.¹⁸ All preparations were manufactured by Stallergènes SA. Because of the role of bacterial products, such as endotoxin, in the control of T_H cytokines, and because the recombinant was expressed in *E coli*, the concentration of endotoxin was measured in the 3 active products. The content of endotoxin was 1.07 EU/mL in birch extract, 1.18 EU/mL in nBet v 1, and <0.05 EU/mL in rBet v 1. The biological activity of rBet v 1, nBet v 1, and birch pollen extract was compared in a pilot skin test study.¹⁹ The placebo preparations consisted of 3 preparations of histamine dihydrochloride (0.0005 mg/mL, 0.005 mg/mL, and 0.05 mg/mL). Birch pollen counts were measured locally in Copenhagen, Gothenburg, Milan, Strasbourg, and Vienna. The usual dates of the typical birch pollen season were March to May (Austria, France, and Italy), April to June (Denmark), and mid-April to June (Sweden).

The patients were given rescue medication (oral, nasal, and ocular antihistamines, nasal corticosteroids) for relief of moderate-to-severe rhinoconjunctivitis symptoms; in the case of insufficient response to the listed medicines, the investigator could provide oral corticosteroids.

Efficacy assessment

Primary outcome variables included daily average rhinoconjunctivitis total symptom score and daily average rescue medication score during the birch pollen seasons. The pollen seasons were determined by pollen counts assessed in each study center and were defined as the period between the first and last day with at least 40 grains/ $\text{m}^3/24$ h.²⁰ The pollen counts were obtained from the European Aeroallergen Network (available at <http://www.univie.ac.at/ean>).

The rhinoconjunctivitis total symptom score was defined as the sum of 8 daily scores including symptoms of rhinitis (itching, sneezing, nasal discharge, congestion) and conjunctivitis (itching, tearing, foreign body sensation, redness). The patients recorded daily, on a diary card, the severity of each symptom on a 4-point scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe).

The daily rhinoconjunctivitis total symptom score ranged from 0 to 24. Rescue medication score was calculated arbitrarily as follows: 2 points for every antihistamine tablet, 0.5 point for each puff of nasal antihistamine and each eye drop of antihistamine, 1 point for each puff of nasal corticosteroid, and 18 points for every tablet of oral corticosteroid (20 mg prednisolone) taken.

Secondary outcome variables were changes in skin reactivity to 50 $\mu\text{g/mL}$ rBet v 1 or nBet v 1 and standardized birch pollen extract containing 50 $\mu\text{g/mL}$ Bet v 1 performed before treatment and after the first and second year of treatment.

Further secondary outcome variables were changes in birch pollen, Bet v 1 and Bet v 2 IgE, IgG, IgG₁₋₄, IgM, and IgA antibody levels. Birch pollen and

rBet v 1 specific IgE antibody levels and rBet v 1 specific IgG levels were quantified by ImmunoCAP measurements (Phadia, Uppsala, Sweden). Birch pollen, rBet v 1 and rBet v 2 specific IgG₁₋₄, IgE, IgA, and IgM levels were measured by ELISA.²¹ Plate-to-plate variabilities were normalized by including reference sera on each plate.²²

Safety assessment

Safety parameters included frequency, severity, and relationship to treatment of adverse events, including immediate and delayed responses.

Statistical analysis

Daily average symptom and rescue medication scores during the pollen seasons were described for each treatment group as means $\pm$ SDs. Each response variable was analyzed by ANOVA including treatment and centre as factors. The treatment effect was estimated by using the differences of the least-square means between each pair of treatments with their corresponding 2-sided 95% CIs. Pairwise comparisons were performed by applying a Bonferroni adjustment to ensure a type I error no more than 5%. A nonparametric approach was used (ANOVA on the ranks) in case of deviation from normality.

Changes in skin reactivity were analyzed in a similar way to symptom scores. Comparisons of immunologic parameters were performed by using Wilcoxon 2-sample tests.

An exploratory statistical analysis was performed to measure the number needed to treat as an assessment of treatment effect.²³ A greater than 30% reduction of symptoms compared with baseline was defined as the cutoff, a limit often recommended to assess the efficacy of subcutaneous immunotherapy.²⁴ Adverse events were summarized descriptively by treatment group.

RESULTS

Patients

The patients are described in a consort diagram (Fig 1). Table I shows that the 4 treatment groups were well balanced in terms of sex, age, birch pollen-specific IgE levels (with 1 patient in the nBet v 1 group with 0.4 specific IgE being a protocol deviation), skin sensitivity to birch, and coexisting allergic symptoms. Polysensitization was present in 81% of patients and asthma in 16% to 28%. This rate of polysensitization is high because exclusion at screening involved only clinically pertinent reactivity. Examination of individual diary cards showed that no patient left the pollen areas during the pollen season.

Improvement of rhinoconjunctivitis and medication scores in the actively treated groups during the 2004 and 2005 pollen seasons

The daily birch pollen counts in the 5 centers for the 2004 season are shown in Fig 1, A. In all centers, pollen counts exceeded 200 grains/ m^3 . During the first pollen season, rhinoconjunctivitis symptom and medication scores were already significantly lower in the 3 treated groups than in the placebo group (Fig 2, B and C). The mean ($\pm$ SD) rhinoconjunctivitis total symptom score for the placebo group was 6.60 ± 4.82 . The adjusted mean differences versus placebo with nBet v 1, rBet v 1, and birch pollen were 3.85 (95% CI, 1.55-6.16; $P = .0006$), 3.32 (95% CI, 1.07-5.57; $P = .0002$) and 3.41 (95% CI, 1.10-5.73; $P = .0024$), respectively (Table II). The percentage improvements in symptom score compared to placebo were 58.3% for nBet v 1, 49.4% for rBet v 1, and 48.0% for birch pollen. The increase in clinical scores in the last 3 days probably corresponds to a decrease in medication use.

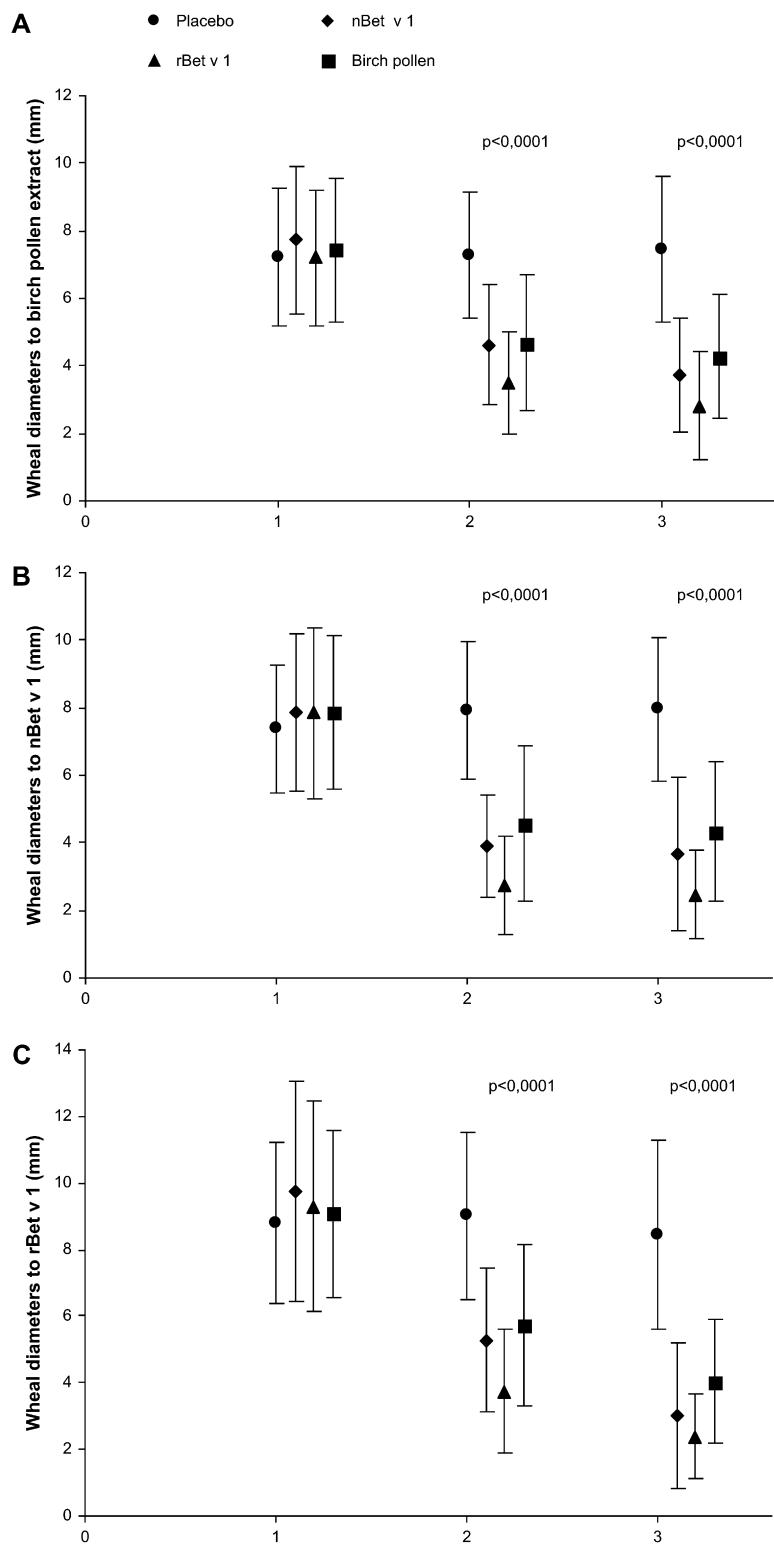
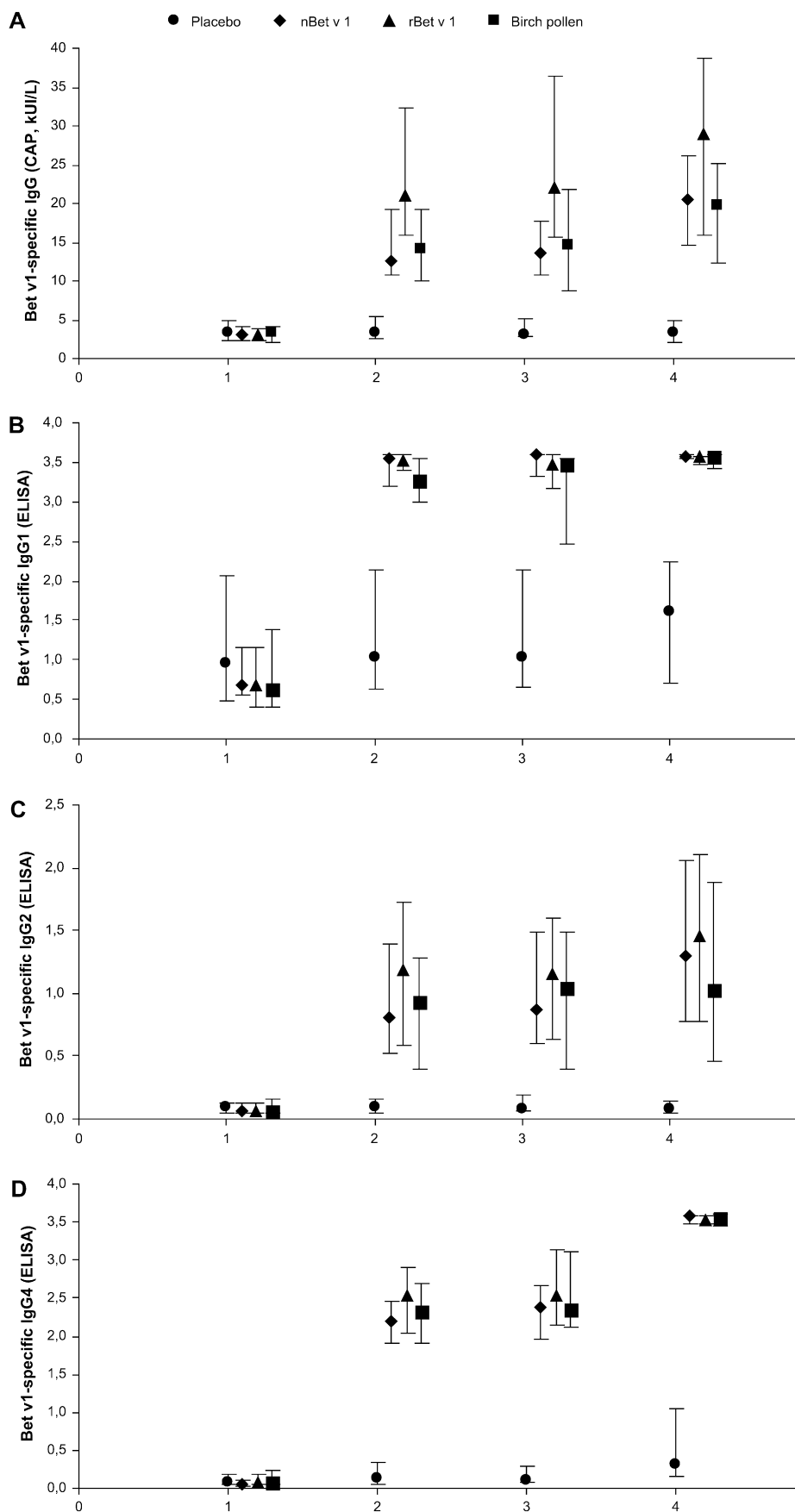


FIG 3. Effect of treatment on skin sensitivity to birch pollen extract (**A**), nBet v 1 (**B**), and rBet v 1 (**C**). Mean wheal diameter (mm) $\pm$ SD at different time points (1, before treatment; 2, after 1 year of treatment; 3, after 2 years treatment). *P*, Comparison of changes from baseline between placebo group and each active treatment group after 1 and 2 years of treatment.



The mean (SD) rescue medication score for the placebo group was 2.96 (4.04). The adjusted mean differences versus placebo were 1.93 (95% CI, 0.35-3.50; $P = .0025$) for nBet v 1, 1.86 (95% CI, 0.32-3.39; $P = .0011$) for rBet v 1, and 1.90 (95% CI, 0.33-3.48; $P = .0063$) for birch pollen, respectively. The percentage improvements in rescue medication score compared with placebo were 63.5% for nBet v 1, 64.2% for rBet v 1, and 69.9% for birch pollen.

No significant differences in rhinoconjunctivitis total symptom scores or rescue medication were observed between the 3 actively treated groups. Similar results were obtained during the second year of treatment (2005; Fig 2, D-F). In this season, the 8 patients from Milan were excluded from the analysis because of a lack of relevant pollen exposure. These figures showed a very important pollen peak in Sweden (Fig 2, D), which did not induce an increase in symptom scores (Fig 2, E), even in the placebo group; however Sweden contributed only 1/3 of the patients. It should be noted, however, that this very large Swedish pollen peak did induce a marked increase in medication scores (Fig 2, F).

When considering the rhinitis and conjunctivitis scores separately, the active groups differed significantly from the placebo group for both seasons (data not shown).

Active treatment reduces skin sensitivity to rBet v 1, nBet v 1, and birch pollen extract

Fig 3 shows the skin sensitivity to birch pollen extract (Fig 3, A), nBet v 1 (Fig 3, B), and rBet v 1 (Fig 3, C) before treatment and after 1 and 2 years of treatment. The skin test results from enrollment were similar to the skin sensitivity before treatment and are not shown. Immunotherapy with each active treatment strongly and significantly reduced birch pollen specific skin reactivity as well as rBet v 1 and nBet v 1 skin reactivity ($P < .0001$) after 1 and 2 years of treatment compared with the placebo group, which showed no change in skin reactivity. Interestingly, rBet v 1 treatment induced a significantly greater decrease in skin sensitivity to birch pollen extract than treatment with nBet v 1 or birch pollen extract ($P < .05$).

Effects of treatment on the development of Bet v 1-specific antibody levels

The development of Bet v 1-specific IgG and IgG subclass levels in the 4 groups is shown in Fig 4. A marked increase in Bet v 1-specific IgG antibody levels was observed only in the 3 actively treated groups (Fig 4, A). This represents the sums of the increases in Bet v 1-specific IgG₁, IgG₄, and IgG₂ levels together (Fig 4, B-D). No changes in Bet v 1-specific IgE, IgA, and IgG₃ were noted to the particular time point when the measurement was done (data not shown).

Effects of treatment on the development of Bet v 2-specific antibody levels

Three new sensitizations to Bet v 2 appeared in the birch pollen-treated group, and for 1 already sensitized patient, the

level of Bet v 2-specific IgE increased after treatment. In addition, compared with the rBet v 1-treated group, higher levels of birch-specific IgG₁ were observed in the birch pollen-treated group (data not shown). Similarly, Bet v 2-specific IgG₁ levels tended to be higher in the birch pollen-treated group than in the rBet v 1-treated group. The 3 newly Bet v 2-sensitized patients in the birch pollen-treated group presented a marked increase in Bet v 2-specific IgG₁ after treatment (with levels 9, 19, and 41 times higher after treatment). No new sensitizations to Bet v 2 were observed in the nBet v 1-treated and rBet v 1-treated groups. Two patients initially sensitized to Bet v 2 in the rBet v 1 group did not have any increase in Bet v 2-specific IgE after treatment.

Safety

The frequency of local or systemic reactions was similar for all groups, and most occurred during the build-up phase (Table III). Two patients in each of the 3 active treatment groups experienced serious adverse events (SAEs); 1 of them had 2 SAEs. Most SAEs were not treatment-related: appendicitis, endometriosis, hemorrhoids, hysterectomy, stroke, and headache. Only 1 SAE was treatment-related: systemic anaphylactic reaction (urticaria with drop in blood pressure) in the nBet v 1 group, which required dose reduction but did not require discontinuation. One SAE (stroke in a patient in the rBet v 1 group) led to discontinuation of treatment.

Immediate local reactions (itching, pain, warmth, or swelling) were reported in all groups, whereas delayed local reactions were more frequent in the 3 active treatment groups. A local reaction caused by histamine contained in the placebo was reported by 7 patients from this group because of the size of reactions. There is no explanation for the high number of local reactions with rBet v 1.

DISCUSSION

This is the first study to demonstrate that immunotherapy with a single recombinant allergen is effective for the specific treatment of allergy compared with a complex natural allergen extract. Treatment with rBet v 1 reduced both symptoms of birch rhinoconjunctivitis and birch pollen-induced skin reactivity throughout the 2 years of treatment. The improvement was similar to that achieved with birch pollen extract or nBet v 1 treatment. Clinical improvement with each active treatment was of the order of 50%, which is close to the mean clinical improvement rate of 45% reported for allergen injection immunotherapy for rhinitis.²⁴ This report integrates different levels of clinical results with some patients having a good response and others a very poor response. Fluctuations in pollen count from one year to another and from one place to another are taken into account in these observations. As a measure of absolute efficacy, the number needed to treat calculation gave pertinent results. Treatment with rBet v 1 gave a number needed to treat of 3.9 with a 95% CI of 3.6 to 4.1; with nBet v 1, the number needed

FIG 4. Effect of treatment on Bet v 1-specific IgG (A), IgG₁ (B), IgG₂ (C), and IgG₄ (D). Antibody levels (medians associated with first [Q1] and third quartiles [Q3]) for the various treatment groups are shown at different time points (1, before treatment; 2, after the first treatment season; 3, after 1 year of treatment; 4, after 2 years of treatment).

TABLE III. Most frequent (at least 10%) possible or probable related treatment-emergent adverse events (safety population; N = 134)

	Placebo (n = 36)	nBet v 1 (n = 32)	rBet v 1 (n = 33)	Birch extract (n = 33)
TEAE	46	37	79	65
Rhinitis	13	12	16	20
Sneezing	4	1	4	4
Cough	1	0	1	5
Conjunctivitis	7	12	6	17
Eye pruritis	14	2	4	4
Local reactions	7	10	48*	15

TEAE, Treatment-emergent adverse event.

*Thirty-two of these were local swelling.

to treat was 3.5 (95% CI, 3.3-3.8); and with birch extract, it was 3.3 (95% CI, 3.0-3.5).

No serious or systemic adverse events related to rBet v 1 treatment were observed, which may be a result of the precision and consistency of the treatment. Only 1 SAE was considered to be treatment-related, and this occurred in the nBet v 1 group. Local reactions were comparable in all groups. The maintenance dose of 15 µg corresponds to the established amount of major allergen (range, 5-20 µg) to be injected to generate efficacy¹⁵; it also corresponds to the amount of Bet v 1 injected in a previous highly effective clinical trial.²⁵

This study confirms that immunotherapy with Bet v 1 reduces skin test responses to Bet v 1, an effect that has also been described with grass pollen²⁶ and tree pollen extract.²⁷

Clinical improvement and reductions in skin sensitivity were observed simultaneously with an intense induction of Bet v 1-specific IgG antibodies, mainly those of the IgG₁, IgG₄, and IgG₂ subclasses. No relevant induction of allergen-specific IgA, IgM, and IgG₃ antibodies was noted. No data were available concerning the production of tolerogenic cytokines, but the lack of induction of allergen-specific IgA antibodies tends to suggest that transforming growth factor beta was not induced.^{28,29} The pattern of induced Bet v 1-specific antibodies was similar to that observed in previous studies conducted with natural extracts,³⁰ recombinant grass pollen,³¹ hypoallergenic allergen derivatives,²¹ and the synthetic nucleotide (CpG)-conjugated ragweed (*Ambrosia artemisifolia*) Amb a 1 allergen.³²

A recent publication by Pree et al³³ reported specific Bet v 1 antibodies in patients treated with hypoallergenic recombinant Bet v 1 derivatives. The IgG antibody responses described in this study were similar to those found in the rBet v 1 study.

In conclusion, this study demonstrates that a recombinant allergen is effective in the treatment of allergy. This vaccine can be produced with a high level of purity and reproducibility by recombinant DNA technology and has a number of advantages over treatment with a complex allergen source, including the avoidance of unnecessary induction of IgE against new components. If these results are confirmed in phase III studies, this approach might also be appropriate for allergens other than birch.

Clinical implications: The proof of concept of specific immunotherapy with a recombinant allergen is a cornerstone to the further development of recombinant vaccines and use in immunotherapy practice.

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