

Cross-reactivity between deciduous trees during immunotherapy. I. *In vivo* results

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Summary

Thirty-nine children with rhinoconjunctivitis due to birch pollinosis were given immunotherapy for 3 years with a potent, purified pollen preparation made from either birch alone or from a mixture of birch, alder and hazel. The therapy was evaluated with self-evaluation after each season, daily symptom score during the season, conjunctival provocation test and skin-prick test. All children but one considered that their condition had been improved by the treatment. Symptom scores decreased slightly more in the group treated with the mixture than in the group treated with birch only due to symptoms decreasing faster in the mixture treated group after each pollen peak ($P < 0.001$). The sensitivity of the conjunctiva and the skin decreased significantly in both groups but without any significant differences between the groups. After 3 years the conjunctival sensitivity correlated strongly to eye symptoms during the season. Immunotherapy with a mixture of birch, alder and hazel appears to be at least as effective as a preparation made from birch only when treating birch pollinosis even in a region where birch is totally dominating among the pollens from deciduous trees.

Introduction

There is a well known cross-reactivity among antigens from pollens of different deciduous trees as well as among grasses of the Gramineae family [1–5]. Although not complete, this cross-reactivity could possibly be utilized to facilitate the diagnosis and treatment of pollen allergy by using a well defined allergen preparation made from only a single pollen source within each group of plants. The aim of this investigation was to compare the efficacy of immunotherapy (IT) with either a birch pollen preparation (B) or a mixture of birch, alder and hazel (M) for treatment of deciduous tree pollinosis. The study was done in an area of Scandinavia where oak is seldom found and hazel does not grow.

Materials and methods

Patients

Forty children with hayfever due to birch pollinosis were included. They had all positive case history [6], skin-prick test (SPT) [7] and conjunctival provocation test (CPT) [8] and were living within 20 km of the hospital of Umeå. Although it was not an inclusion criterion, all children had IgE antibodies in serum against birch pollen of class 1 or more according to the Phadebas RAST® system (Pharmacia AB, Uppsala, Sweden). In addition to the birch pollinosis, some of the children had a minor sensitivity to other allergens, mainly grass pollens and animal danders. Children with perennial rhinitis or asthma, children with severe asthma during the birch pollen season or with significant allergy other than sensitivity to deciduous trees were excluded.

The children were allocated by one of us (S.D.) into pairs based on symptom score, CPT, SPT, age and sex without any other knowledge of the patients. Within each pair one of the children was randomly assigned to treatment with a birch pollen preparation and the other one with a mixture of birch, alder and hazel pollen allergen preparation. The extracts were delivered in individually coded vials. The other author (C.M.) had all contacts with the patients and also made the clinical evaluations before breaking the code. Those children who were treated with the birch pollen preparation constituted the B group and those treated with the three-tree mixture the M group. One of the patients dropped out of the study before the start of IT. Results were thus obtained from thirty-nine of the forty children, nineteen pairs being available for the statistical analysis. For details of the two treatment groups see Table 1.

The children and their parents were fully informed regarding the purpose and design of the study and the parents of all children gave their written consent. The investigation was approved by the Ethics Committee of Umeå University Hospital.

Table 1. Pretreatment data on the two treatment groups

Treatment group	Number of patients	Mean symptom score	Mean CPT	Mean SPT	Mean age (range)	Sex M/F
Birch	19	93	3.05	3.40	11.6 (6-16)	12/7
Mixture	20	91	3.10	3.45	11.6 (7-16)	13/7

The figures for CPT and SPT are the ¹⁰log values of the extract potencies as expressed in BU/ml. The SPT value is the extract potency giving the same skin reaction as histamine 1 mg/ml.

Materials

For SPT and CPT against birch and alder freeze-dried, purified and biologically standardized allergen preparations were used (Pharmalgen®, Pharmacia AB). The oak preparation was a biologically standardized freeze-dried crude extract. (Pharmacia AB). For all tests, except for the last CPT in 1983 when new batches of birch and alder were used, the same batches of birch, alder and oak were employed. The potencies and the compositions of the new batches were the same as for the previous ones [9]. Albumin diluent® (0.03% human albumin in 0.9% NaCl with phenol 0.4% Pharmacia AB) was used as negative control and histamine chloride (1.0 mg/ml or 5.43 mmol/l) as positive.

Pollen preparations with the same composition as for the *in vivo* tests were used for IT. The mixture contained equal amounts in biological units (BU) of birch, alder and hazel preparations. All extracts for IT were purified and delivered freeze-dried. The extracts were reconstituted with Albumin diluent® during initial treatment and with Depot diluent® (0.2% aluminium hydroxide) during maintenance IT.

Methods

The SPT were done just before the start of IT and 3 years later. On each occasion nine concentrations were tested, increasing stepwise in $0.5^{10}\log$ steps, i.e., for birch and alder 3.2; 10; 32 . . . up to 32 000 BU/ml and for oak 200; 640; 2000 . . . up to 2 000 000 BU/ml, together with negative and positive controls. The areas of the weals, calculated with a digitizer were used for evaluations. During late autumn CPT with birch, alder and oak were performed before the commencement of IT and after 1, 2 and 4 years. In addition CPT with birch were done after 3 months and 3 years of IT. The tests were performed according to Möller *et al.* [8] with successively higher doses of the allergen administered dropwise into the eyes with 20-min intervals on alternating sides until an allergic reaction i.e., congestion and itching, was noted. The test concentrations were the same as used for SPT. On the last provocation, i.e., after 4 years, for birch and alder the concentrations 100 000; 320 000 and 1 000 000 BU/ml and for oak 6 400 000 BU/ml were added.

The children carried out daily registration of their hayfever symptoms and medication each year from 30 April to 10 June which covered the birch pollen season. Nose and eye symptoms were registered separately using a four-grade scale; no symptoms (0), mild symptoms (1), moderate symptoms (2) and severe symptoms (3). The only medications allowed were clemastine in case of severe hayfever and salbutamol inhaler in case of asthma. If clemastine was used this added one point to the daily symptom score. The maximal daily symptom score was thus seven points. After each season the children reported their impression of whether their symptoms of hayfever were more pronounced, similar to, less or much less pronounced than those in 1979.

During the scoring periods each year pollen counts were registered daily using a Burkard volumetric trap. Thus the main alder season was not registered. However, there are few alder pollens compared to birch in this geographical area [10].

Therapy was started either at the end of 1979 or at the beginning of 1980, the two children in each matched pair starting on the same day. The initial IT was given in the hospital as rush therapy with the pollen preparations reconstituted with Albumin diluent®. Every 2 hr increasing doses were given up to five times a day until a clear-cut allergic reaction was noted. The first depot injection was given 1 week after the first injection in a dose equivalent to the highest earlier tolerated. Maximum tolerated doses were then given with successively longer intervals up to every 4 weeks. Treatment was not given during the birch pollen season i.e., during 6 weeks every year. The therapy was stopped after 3 years (Fig. 1). Side effects were continuously recorded.

Statistical methods

For calculations the potencies of allergen preparations and the areas of weals were logarithmic transformed and pollen counts square root transformed. To compare the symptom scores with the pollen counts a dynamic model was used by which it was

possible to evaluate the instant effects of a pollen peak on symptom scores and the duration of symptoms. The model for this study can be expressed as:

$$Y_{ki}(t) = \mu + \alpha_{ki} + \beta_0 Y_{ki}(t-1) + (\beta_1 - \beta_0)k Y_{ki}(t-1) + \tau_0 P(t) + (\tau_1 - \tau_0)k P(t) + \varepsilon_{ki}(t)$$

where

$Y_{ki}(t)$ = symptom score for patient n :o i , day t , in treatment group k , $i = 1, \dots, n$,
 $t = 1, \dots, 42$, $k = 0$ (birch group) or 1 (mixed group)

μ = overall average symptom score

α_{ki} = individual variation

β_k = duration rate

τ_k = instant impulse response

$P(t)$ = pollen counts

$\varepsilon_{ki}(t)$ = independently and identically distributed random errors with zero means.

The model is described by Broström & Möller (submitted). Other comparisons between groups were done with paired t -tests. Thus, only complete pairs could be analysed. Pearson product-moment correlation coefficient was used to calculate significances of correlations. To estimate changes in skin sensitivity tested by SPT the method of parallel line assay [11] was used. All tests were two-tailed.

Results

The pollen counts 1979–1983 are shown in Fig. 1. The dominating pollen was birch

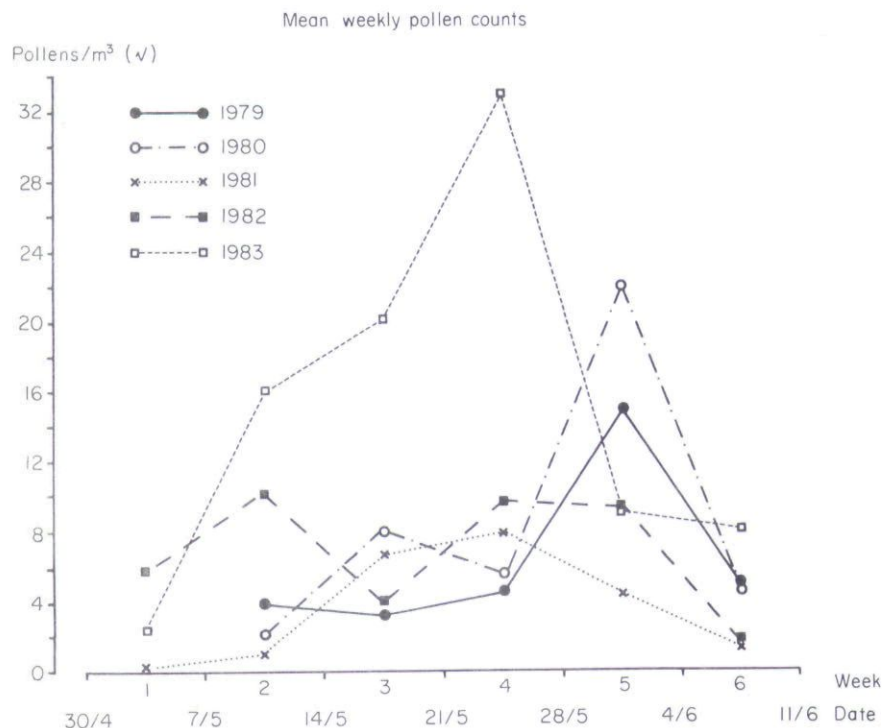


Fig. 1. Mean pollen counts (square root transformed) for deciduous trees in Umeå (1979–1983).

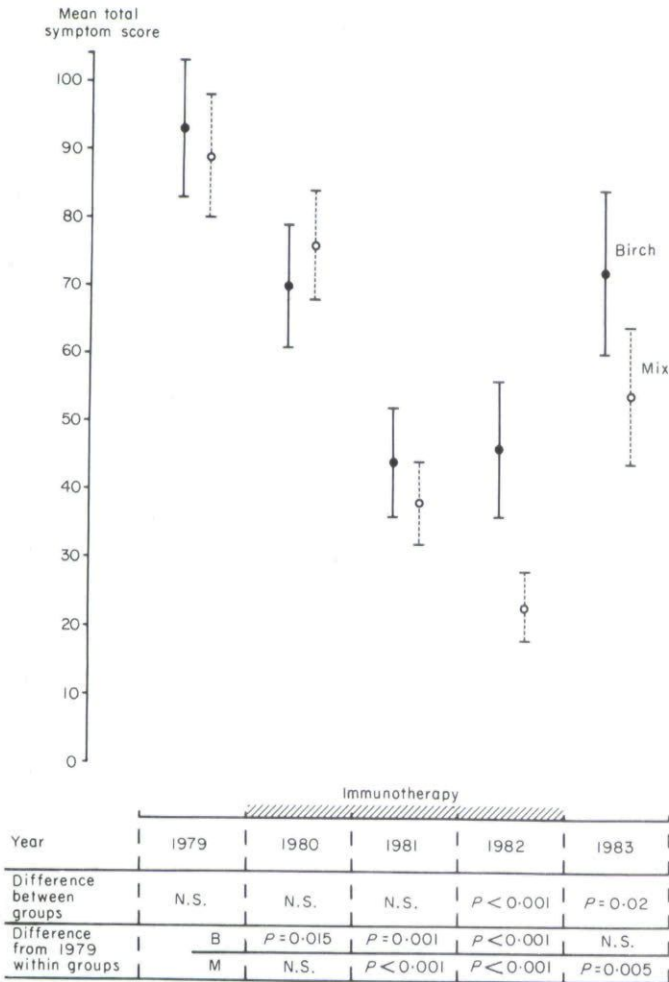


Fig. 2. Total symptom scores 1979–1983.

although some alder, willow and aspen pollen were found. According to the self evaluation after each season all of the children with one exception considered their condition to have improved from 1981 and onwards compared to 1979. The child who did not improve showed the lowest sensitivity as measured with SPT, CPT and RAST before IT.

Total symptom scores did not differ between the groups in 1979, 1980 and 1981. In 1982 (after 2.5 years of therapy) and in 1983 (3–6 months after the cessation of IT) the symptom scores were significantly lower in the M group than in the B group ($P < 0.001$ and $P = 0.04$ respectively). Within each group total symptom scores decreased significantly from 1979 i.e., before IT, to 1981 and 1982, i.e., after more than 1 year of IT and during years with similar pollen counts as 1979 (Fig. 2).

The dynamic analysis for 1982 and 1983 revealed that both groups responded in a similar manner to every pollen peak but the time for the symptoms to disappear was longer in the B group ($P < 0.001$ both years; Figs 3 and 4). Each year symptom scores

from the nose were more than twice the scores from the eyes. The proportions of scores from the nose and the eyes as well as clemastine medication were similar in the two treatment groups.

The conjunctival sensitivity decreased from 1979 (before treatment) to 1983 (after treatment) for all the three tree pollens ($P < 0.001$; Fig. 5). There were no significant differences in this respect between the treatment groups. The CPT results 1979 and 1980 correlated poorly to symptoms 1982 and 1983. However, during 1983 a high conjunctival sensitivity was associated with high eye symptom scores. In the B group CPT for birch correlated better with eye symptoms ($P < 0.01$) than CPT results using alder and oak extracts. In contrast, in the M group CPT for alder and oak correlated better with clinical symptoms ($P \leq 0.01$) than CPT for birch. The skin sensitivity for birch, alder and oak decreased ($P < 0.05$) in a similar manner in both treatment groups. There were only weak correlations between the decreases in SPT on one hand and the symptom scores 1982 and 1983 and CPT results on the other.

Before the start of IT thirteen children had a mild asthma during the leafing season. During IT the symptoms of asthma disappeared in five, improved in two, was similar in two and worsened in four children. Another four children developed asthma during the study period. All these children as well as those in whom the asthma worsened also had wheezing outside the season. In one child asthma and arthritis appeared after 1.5 years of therapy. Even though the symptoms were probably induced by a viral infection IT was discontinued. The joint symptoms promptly disappeared. Generalized reactions such as urticaria and asthma in connection with the injections were seen in five patients during rush IT and in eleven patients during maintenance therapy. No anaphylactic shock was seen. Local reactions such as swelling and itching at the site of the injection were recorded in all but one of the children on at least one occasion. These reactions lasted for more than 1 week in eleven children. For five of these children the itching

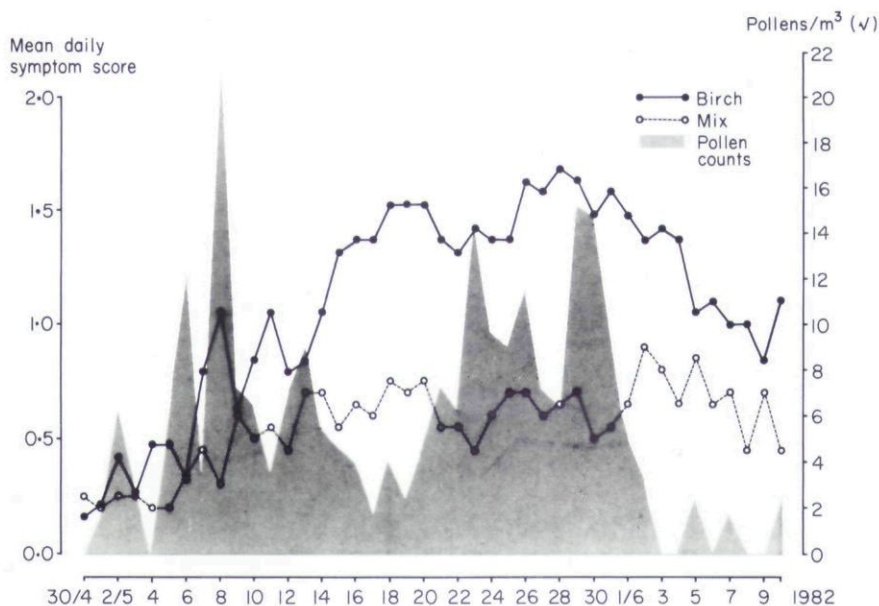


Fig. 3. Mean daily symptom scores 1982.

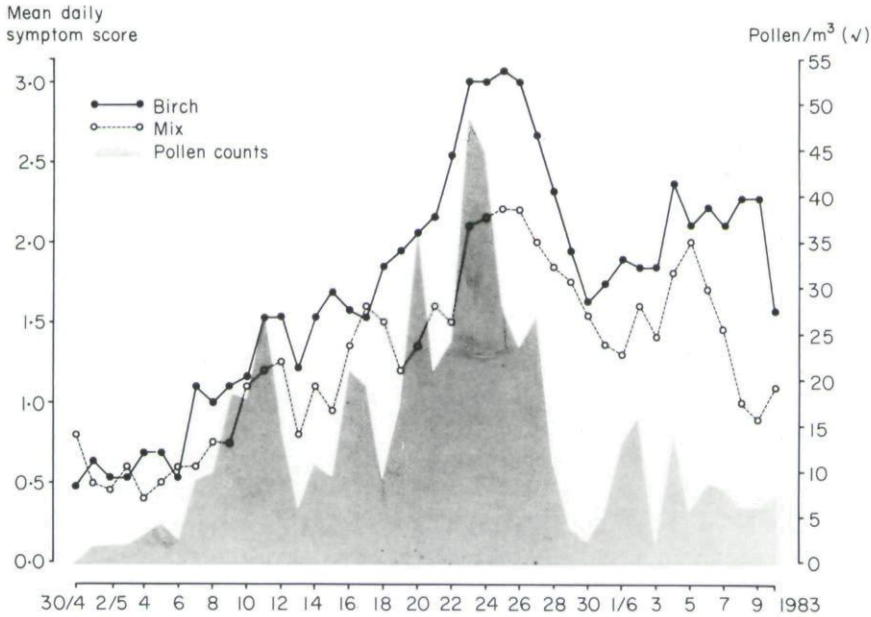


Fig. 4. Mean daily symptom scores 1983.

lasted for more than 6 months after the termination of IT.

Discussion

Immunotherapy with the mixed pollen extract seemed to yield better results than the treatment with the birch pollen extract as measured by daily symptom scores. This

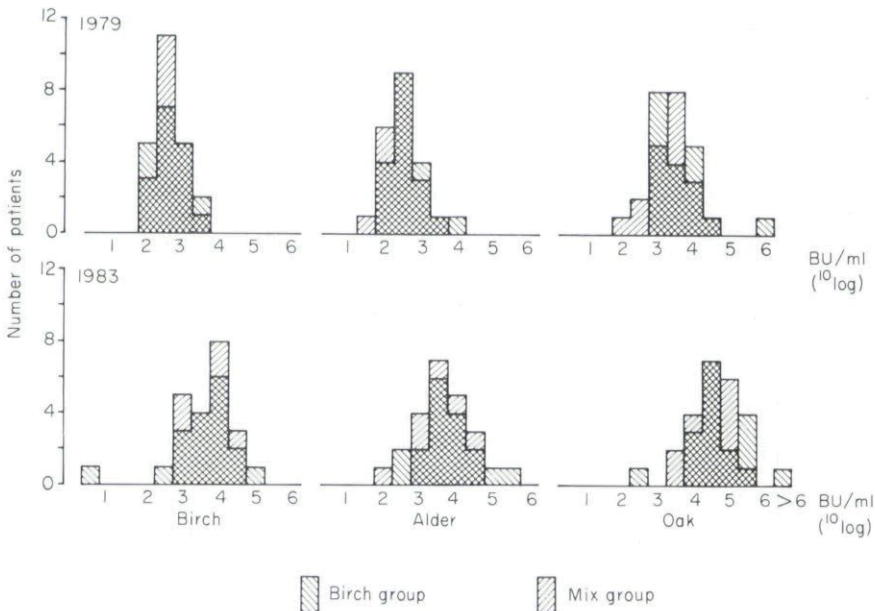


Fig. 5. CPT results before (1979) and after IT (1983).

finding was surprising since very few pollens except birch were found in the pollen trap. Possibly the alder season starting in late April, could cause higher symptom scores for the group treated with birch only by means of a priming mechanism with a carry-over effect. But this explanation is unlikely as the difference between the treatment groups was less in the beginning of the season than towards the end and as the difference was not influenced by pollen counts. The reason for the differences between the treatment groups therefore remains unexplained.

Symptom scores were higher in 1983 than in 1982 in both treatment groups. The most likely explanation for this finding is the much higher overall pollen count during the 1983 season, although the possibility of a decreasing effect of IT after completion of the treatment has stopped cannot be excluded.

Conjunctival provocation tests and SPT were performed with birch, alder and oak in all patients, i.e., with one pollen given to both groups, one pollen given only to one of the groups and one pollen not used for therapy in any of the patients. Conjunctival and skin sensitivity decreased during IT and the decrease was similar in both groups. The similar decrease in sensitivity to alder is surprising as only one group was treated with a preparation containing alder. The CPT results for birch (but not for alder or oak) correlated with eye symptom scores in the birch group whereas in the mixture group CPT results using alder and oak but not birch correlated with the eye symptom scores. This indicates that supplementary treatment with alder may influence the symptoms even during a season dominated by birch pollen.

The skin-prick test is a precise method when performed by a trained nurse [7]. Like CPT, SPT failed to reveal any differences between the two forms of IT. Thus, only the daily symptom scoring indicated that treatment with the mixed tree pollen extract was superior to the birch pollen extract. Two studies [12, 13] using grass pollens which cross react to roughly the same extent as our tree pollens [1, 3] have shown IT with a purified timothy pollen preparation to be superior to a crude mixture of different grasses. On the other hand in another study a timothy pollen preparation containing eighteen allergens was found to be superior to an extract containing only two of the major allergens [14]. Recently it was shown that a purified five grass pollen mixture preparation was slightly more effective than a purified extract of timothy alone [15]. These latter findings support our results and could indicate that a pollen preparation made from a mixture of closely related species is superior to a preparation made from only one species among both deciduous trees and grasses. In a study from Denmark, with similar aims to ours, no differences were found between the treatment groups after 1 year of therapy [16]. However, in our study the difference between the groups was apparent only after 2.5 years of IT.

In conclusion, IT with a preparation containing a mixture of allergens from birch, alder and hazel gave a better result than an equipotent birch allergen preparation even in a region where birch is the dominating deciduous tree but this difference was small and obvious only after 2 years of treatment. Neither CPT nor SPT prior to IT can predict the outcome of the therapy.

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