

Immunotherapy with partially purified and standardized tree pollen extracts

III. Specific IgE response to the major allergens of alder, birch and hazel pollen during immunotherapy

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Patients allergic to pollen from alder, birch and hazel were hyposensitized during a 3-year period with either birch pollen extract alone ($n = 24$) or a mixture of one or more of alder, birch and hazel pollen extracts ($n = 27$). The effect of the treatment was evaluated by RAST and tandem crossed-radioimmuno-electrophoresis (tandem-CRIE). The patient's specific IgE response to the major allergens of alder (Aln g I), birch (Bet v I) and hazel (Cor a I and Cor a II), as measured by tandem-CRIE, and the total specific IgE response, measured by RAST, decreased significantly ($P_c < 0.05$) during immunotherapy, irrespective of the extract used during the treatment. There was no significant difference ($P_c < 0.05$) between the two treatment groups. The results obtained indicate either that birch pollen extract alone is adequate in the treatment of the studied patient group or the patients had been sensitized towards birch pollen alone.

Key words: alder; birch; CRIE; hazel; immunotherapy; tree-pollens.

Accepted for publication 11 February 1988

Patients allergic to birch pollen often exhibit additional sensitivity to pollens from alder and hazel (2). The patients' cross sensitivity to tree pollen may originate from the immunochemical partial identity which exists between the major allergens from these pollens (4). Alternatively, tree pollen-allergic patients may be sensitized specifically to more than one pollen source. Immunotherapy of patients allergic to pollens of alder, birch and hazel with a birch pollen extract alone is anticipated to be adequate if the cross sensitivity originates exclusively from the partial identity between the major allergens. Specific sensitization to more than one allergen source would probably require an immunotherapy scheme involving the relevant pollen extracts.

In the present study patients sensitive to alder, birch and hazel pollens were allocated to two

treatment groups, one of which was hyposensitized with a birch pollen extract alone, whereas the other was hyposensitized with a mixture of one or more alder, birch and hazel pollen extracts. The patients' IgE response to the individual major allergens of alder, birch and hazel and the total specific IgE response were measured before and throughout the 3-year hyposensitization period (1981 to 1983 inclusive).

MATERIAL AND METHODS

Pollen extracts

Extracts of *Alnus glutinosa* (Ag), *Betula verrucosa* (Bv) and *Corylus avellana* (Ca) pollens used for immunoelectrophoretic purposes were pre-

pared as described recently (4, 5). Extracts of the different pollens used for immunotherapy and RAST were obtained from Allergologisk Laboratorium, ALK, Copenhagen, Denmark (6).

Rabbit antibodies

Rabbit antibodies against the pollen extracts were raised as described elsewhere (4, 5). Immunization and subsequent isolation of the immunoglobulins were performed essentially as described by Harboe & Ingild (3).

Patients and clinical design

The selection of alder, birch and hazel pollen-allergic patients and their allocation to two treatment groups are described in detail elsewhere (6). Briefly, previously untreated patients with seasonal allergy towards alder, birch and/or hazel pollens were allocated to two treatment groups, one receiving immunotherapy with mixtures of alder, birch and hazel pollen extracts (Group I (ABC)) and the other with birch pollen extract alone (Group II (B)). The patients were allocated to the treatment groups according to their individual response in CRIE. Whenever possible, patients with identical or approximately identical CRIE response patterns were allocated to different treatment groups (6). Serum samples from each of the patients were obtained before and throughout the treatment period; in all, eight samples from each of the patients were drawn.

RAST

RAST was performed as described (1, 9) at the Diagnostic Laboratory, ALK, Copenhagen, Denmark. The paper discs were coupled with either alder pollen extract Batch 002 ALK (coupling concentration: 68 µg/disc), birch pollen extract Batch 909 ALK (coupling concentra-

tion: 3 µg/disc) or hazel pollen extract batch 918 ALK (coupling concentration: 25 µg/disc). Allergen-specific IgE activity in the patients' sera was calculated as:

$$\% \text{ CPM (B)} = \frac{\text{CMP (B)} - \text{CPM (N)}}{\text{CPM (T)}} \cdot 100$$

where CMP (B) denotes the counts per minute bound to the paper discs after incubation with the patients' sera and subsequent incubation with ^{125}I -anti-IgE, and CMP (T) is the total counts per minute of ^{125}I -anti-IgE added. CMP (N) is the counts per minute measured using a 1000 donor serum pool in place of the patients' sera.

Immunoelectrophoretic methods

Tandem crossed-radioimmunoelectrophoresis (tandem-CRIE) was performed as described recently (4). Briefly, tandem crossed-immunoelectrophoresis was run on 7 × 5 cm glass plates in 1% (w/v) HSA agarose (Litex, Copenhagen, Denmark) (0.073 M TRIS, 0.024 M barbitale, 0.0006 M calcium lactate, pH 8.6) at 15°C; antigen application; 5.0 µg Ag, 5.0 µg Bv and 10.0 µg Ca were applied, respectively, in three wells punched in the agarose 1 cm apart (along the 7 cm side of the glass plate); first dimension electrophoresis: 25 min, 10 V/cm; second dimension electrophoresis: 16 h, 2 V/cm, with the use of 12 µl/cm² of a 1:1:1 mixture of anti-Ag, anti-Bv and anti-Ca in the antibody-containing gel. Cold-dried, non-stained tandem-CIE plates were next incubated with 0.5 ml serum in 8.0 ml of 0.067 M phosphate buffer, pH 7.0 for 24 h at 23°C, followed by incubation with approximately 140 nCi of ^{125}I -anti-IgE in 8.0 ml 0.067 M phosphate buffer pH 7.5, 0.3% (w/v) bovine serum albumin, 0.154 M sodium chloride, 0.015 M sodium azide, 0.027 M EDTA for 24 h at 23°C. The plates were washed (three times 20 ml of 0.154 M sodium chloride, 1% (v/v)

Fig. 1. Examples of tandem crossed-radioimmunoelectrophoresis (tandem-CRIE) 10-day autoradiographs obtained using serum samples from individual patients drawn at the time indicated. a: Aln g I; b: Bet v I; c: Cor a I; d: Cor a II.

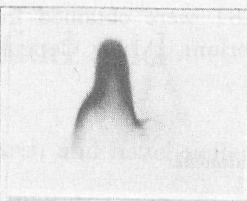
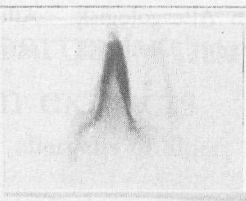
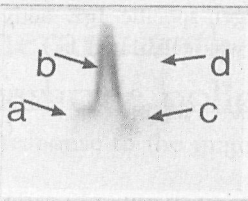
Sample
time

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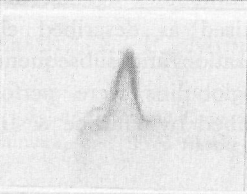
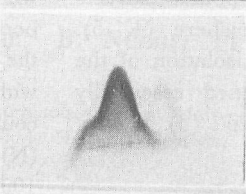
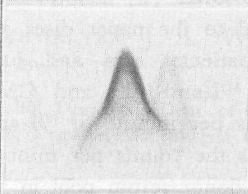
Patient #2

Patient #3

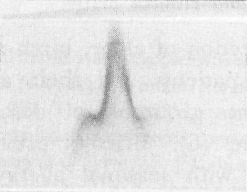
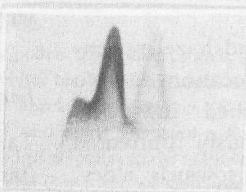
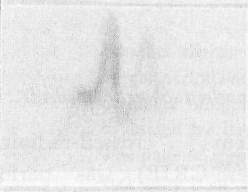
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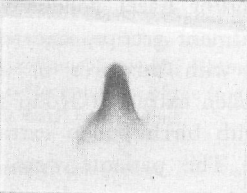
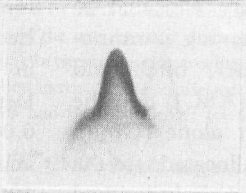
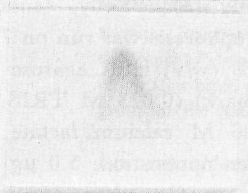
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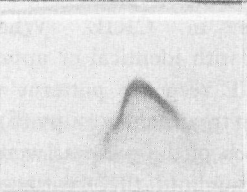
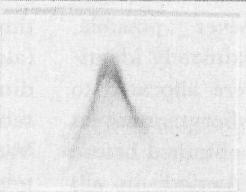
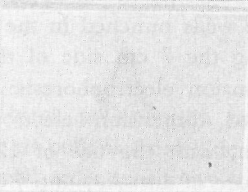
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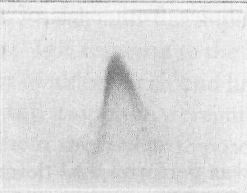
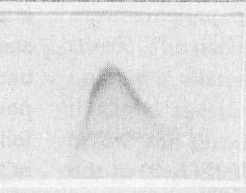
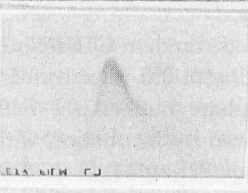
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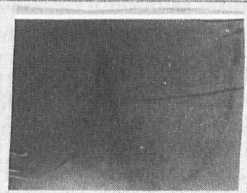
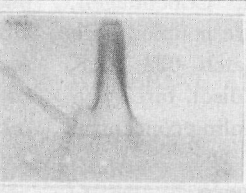
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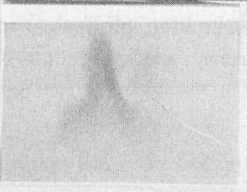
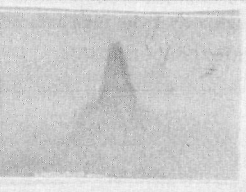
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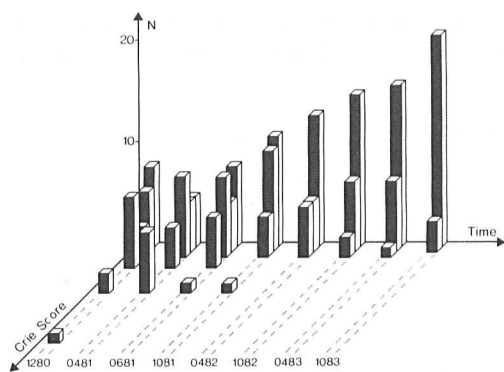
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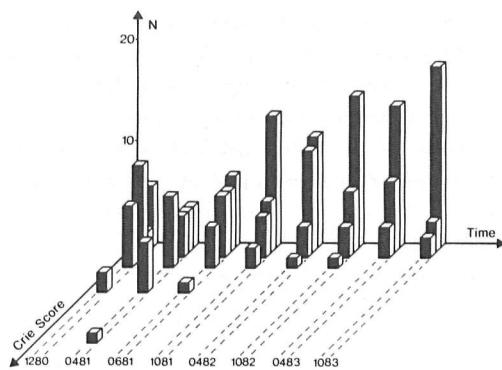
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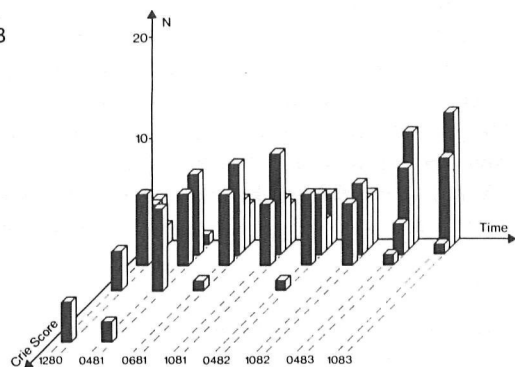
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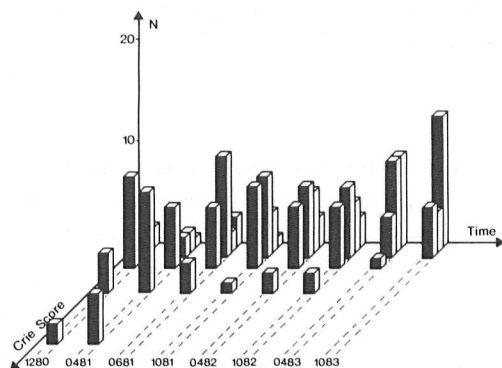
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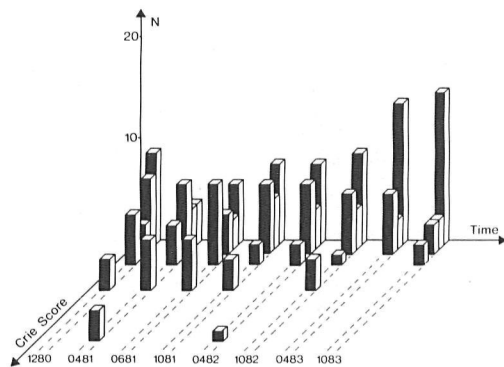
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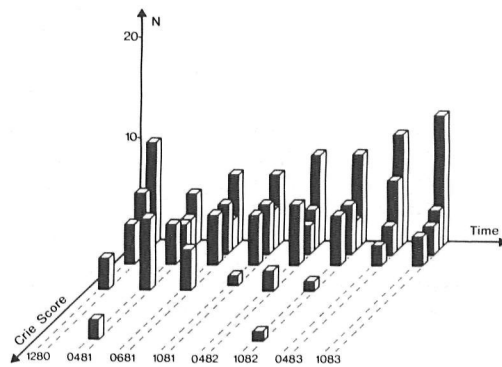
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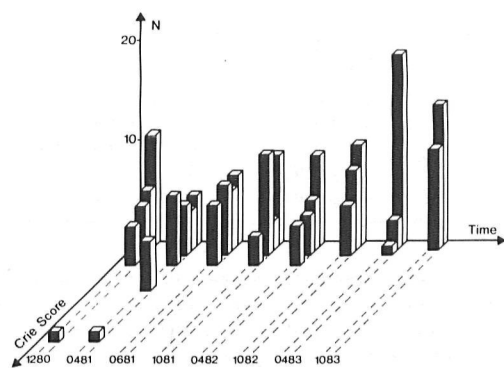
C



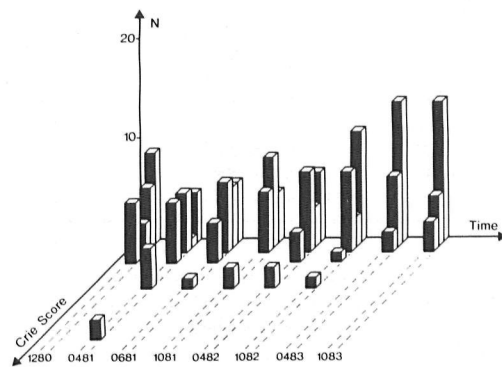
C



D



d



Tween 20), dried, and subjected to autoradiography on Kodak XL-1 X-ray film for 4 and 10 days. The degree of radiostaining of the precipitates was evaluated by visual comparison of the tandem-CRIE autoradiographs with the autoradiographs of Swine anti-rabbit IgG precipitated ¹²⁵I-anti-IgE Mancini rings (SRRID) (10). The radiostained precipitates were assigned a score according to:

N = 512/D

where D is the dilution of ¹²⁵I-anti-IgE in the SRRID-ring with corresponding radiostaining intensity.

Statistics

Wilcoxon matched pairs rank sum test and Mann-Whitney U-test (7) were used to evaluate differences within and between the treat-

ment groups. All two-sided stochastic probabilities < 0.05 were considered significant.

RESULTS

Specific IgE response towards the major allergens

Fig. 1 shows examples of tandem-CRIE autoradiographs, obtained with patients' sera drawn at different times throughout the treatment period (1280-1083). All sera contained IgE which in tandem-CRIE bind to the major allergen of alder (Aln g I), birch (Bet v I) and hazel (Cor a I and Cor a II). Most of the sera exhibited a strong tandem-CRIE radiostaining of Bet v I and an intermediate radiostaining of Aln g I, whereas only a few sera led to a strong radiostaining of Cor a I and Cor a II.

Fig. 2 shows the number of patients in the two treatment groups who responded to the major

Table 1

The mean of the tandem-CRIE scores determined for the major allergens of alder (Aln g I), birch (Bet v I) and hazel (Cor a I and II) throughout the treatment period

< tandem-CRIE score > ± 1.96*SEM				
Time	Group I (ABC)			
	Aln g I	Bet v I	Cor a I	Cor a II
1280	23.2 ± 10.2	82.7 ± 31.2	9.0 ± 3.7	7.7 ± 4.7
0481	25.6 ± 8.5	92.5 ± 27.0	18.3 ± 7.4	14.7 ± 5.5
0681	15.8 ± 5.5	37.2 ± 10.8	13.1 ± 4.2	7.0 ± 2.2
1081	9.4 ± 4.8	32.3 ± 8.1	8.4 ± 3.9	5.9 ± 2.0
0482	6.6 ± 2.5	38.2 ± 12.9	7.9 ± 5.6	5.6 ± 2.4
1082	5.1 ± 1.9	33.3 ± 9.4	8.0 ± 4.2	3.5 ± 1.2
0483	4.0 ± 1.8	14.4 ± 6.0	3.0 ± 1.4	1.5 ± 0.8
1083	3.1 ± 1.1	10.5 ± 3.2	3.6 ± 1.9	2.0 ± 0.7
Group II (B)				
1280	20.7 ± 6.8	74.7 ± 27.0	9.5 ± 4.1	6.7 ± 2.7
0481	32.3 ± 11.9	116.3 ± 33.5	19.5 ± 7.4	17.2 ± 7.1
0681	15.4 ± 5.7	47.5 ± 14.6	11.3 ± 4.4	7.5 ± 2.9
1081	8.6 ± 3.7	43.8 ± 11.6	8.0 ± 3.3	6.8 ± 3.6
0482	7.8 ± 2.9	41.0 ± 13.4	8.6 ± 3.8	8.1 ± 3.5
1082	6.8 ± 2.9	41.9 ± 13.9	9.8 ± 5.6	5.8 ± 2.7
0483	5.2 ± 2.2	16.9 ± 5.7	3.8 ± 1.9	2.4 ± 1.0
1083	3.8 ± 2.0	13.3 ± 4.8	4.2 ± 2.2	2.4 ± 1.2

Fig. 2. The "amount" of major allergen-specific IgE (tandem-CRIE score) determined for each of the patients in the treatment groups (Group I (ABC) and Group II (B)) are plotted against time. The columns indicate the number of patients who obtained the specified tandem-CRIE score at the time indicated. A, a: Aln g I; B, b: Bet v I; C, c: Cor a I; D, d: Cor a II. Upper and lower case letters indicate Group I (ABC) and Group II (B) respectively.

allergens of alder, birch and hazel at the different tandem-CRIE scores indicated throughout the treatment period. Before immunotherapy was initiated (1280) the tandem-CRIE scores were distributed over a considerable range of radio-staining levels, but in the treatment period

(0481-1083) an increasing number of patients exhibited tandem-CRIE scores which became smaller, approaching the zero level at the termination of therapy. Table 1 shows the same development expressed as the mean tandem-CRIE score; the score determined for Aln g I and

Table 2
Wilcoxon matched pairs rank sum test ($2 \cdot P_c$) for tandem-CRIE and RAST-values

Group I (ABC) 1280 versus							
	0481	0681	1081	0482	1082	0483	1083
t-CRIE							
Aln g I	0.384	0.021	0.006	0.001	< 0.001	< 0.001	< 0.001
Bet v I	0.235	0.001	0.002	0.008	0.002	< 0.001	< 0.001
Cor a I	0.001	0.004	0.642	0.174	0.234	< 0.001	< 0.001
Cor a II	0.004	0.505	0.698	0.538	0.107	< 0.001	< 0.001
RAST							
Ag	0.006	0.016	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Bv	0.059	0.004	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Ca	0.308	0.437	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Group II (B) 1280 versus							
	0481	0681	1081	0482	1082	0483	1083
t-CRIE							
Aln g I	0.024	0.008	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Bet v I	0.003	0.003	0.007	0.006	0.007	< 0.001	< 0.001
Cor a I	0.001	0.311	0.650	0.266	0.887	< 0.002	< 0.001
Cor a II	0.001	0.532	0.469	0.444	0.334	< 0.001	< 0.001
RAST							
Ag	0.407	0.199	0.001	< 0.001	< 0.001	< 0.001	< 0.001
Bv	0.753	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Ca	0.835	0.689	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 3
Comparison between Group I (ABC) and Group II (B) Mann-Whitney U-test, $2 \cdot P_c$

	1280	0481	0681	1081	0482	1082	0483	1083
tandem-CRIE score								
Aln g I	0.809	0.459	0.937	0.633	0.586	0.623	0.493	0.867
Bet v I	0.938	0.317	0.337	0.146	0.786	0.504	0.335	0.601
Cor a I	0.985	0.878	0.507	0.828	0.670	0.673	0.699	0.785
Cor a II	0.595	0.687	0.960	0.800	0.339	0.335	0.193	0.924
RAST								
Ag	0.433	0.799	0.828	0.254	0.758	0.874	0.701	0.901
Bv	0.599	0.084	0.792	0.516	0.750	0.946	0.991	0.708
Ca	0.672	0.777	0.850	0.992	0.323	0.460	0.253	0.346

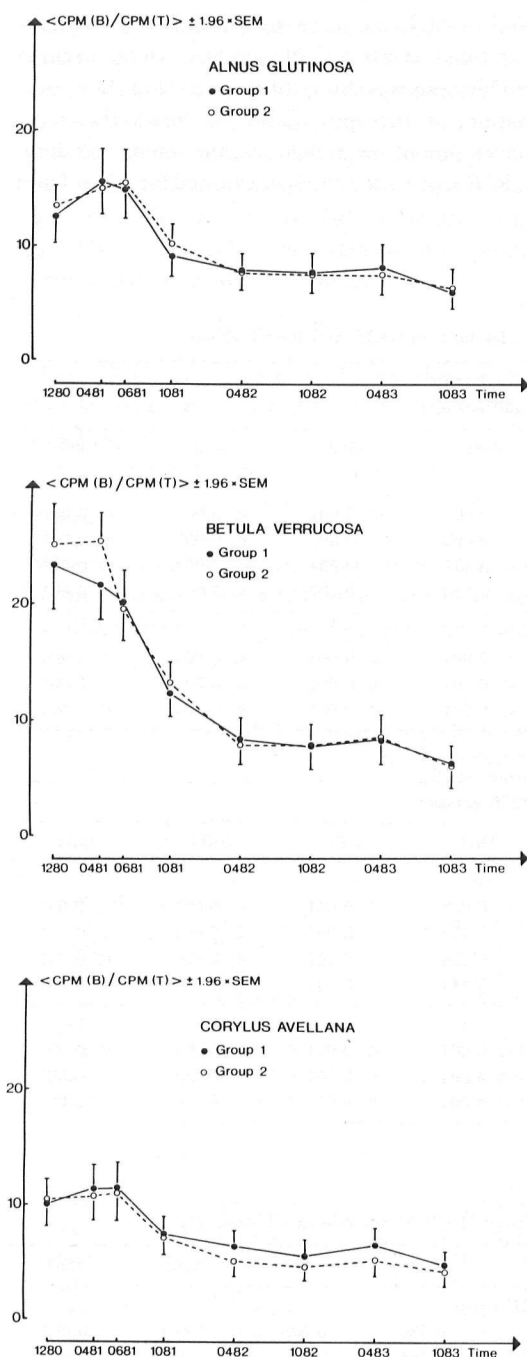


Fig. 3. The mean of the patients' total specific IgE (RAST) ($< \text{CPM (B)/CPM (T)} > \pm 1.96 \cdot \text{SEM}$) in the two treatment groups (Group I (ABC) and Group II (B)), determined by paperdisc RAST are plotted versus time. SEM: standard error of the mean.

Bet v I in treatment Groups 1 (ABC) and 2 (B) decreases statistically significantly at the end of the first treatment year (Table 2) compared with the pre-therapy scores, whereas the scores of Cor a I and Cor a II in both groups first exhibit a significant decrease in the last treatment year (0483) (Table 2). The changes in the tandem-CRIE scores are parallel (Fig. 2, Table 1), and the groups are statistically indistinguishable (Table 3) throughout the treatment period.

Total specific IgE response (RAST)

Fig. 3 shows the mean of the serum-specific IgE values determined by RAST during the treatment period. The RAST data reflect the same pattern as the tandem-CRIE scores: there is a statistically significant decrease in the total specific IgE level towards the allergens of alder, birch and hazel from the end of treatment year 1 and onwards (Table 2) in comparison with the pre-treatment level(s), and the two groups are statistically indistinguishable throughout the immunotherapy period (Table 3).

DISCUSSION

The hyposensitization of patients allergic to alder, birch and hazel pollens either with various mixtures of alder, birch and hazel pollen extracts (Group I (ABC), $n = 27$) or a birch pollen extract (Group II (B), $n = 24$) during a 3-year period (0181-1283) was monitored *in vitro* by RAST (total specific IgE), tandem-CRIE (major allergen specific IgE) and *in vivo* by symptom score (6), skin prick testing (8) and nasal provocation test (8). The mean values of the *in vitro* test parameters determined throughout the treatment period exhibit a statistically significant ($P_c < 0.05$) decrease after approximately 1.5 years of treatment, and the test parameters remained at the lower level for the rest of the treatment period. The same "pattern" was observed for the *in vitro* parameters (6, 8). Further, there is no significant ($P_c < 0.05$) difference between the two treatment groups, whichever parameter is used for comparison.

The results reported here and those described

elsewhere (6, 8), appear to indicate that birch pollen extract alone is suitable for hyposensitization of allergic patients sensitive to alder, birch and hazel pollens. However, since the major allergen of birch pollen, Bet v I, is immunochemically partially identical to alder, Aln g I, and hazel, Cor a I, (4), the patients included in the clinical investigation could be sensitized to birch pollen only and their reaction to the pollens of alder and hazel could then be mediated through identical or partially identical IgE binding epitopes on the allergens. This would lead to the observed result of the clinical investigation. We have found no indications of specific sensitization to alder and/or hazel alone in the studied patient population. Therefore, it is not possible to conclude whether or not birch pollen extract, in general, is adequate for hyposensitization of alder, birch and hazel pollen-sensitive patients. Detailed investigations of the epitope specificities of the patients' IgE are required in order to distinguish between the patients' sensibilization source(s), which may affect the outcome of hyposensitization. "Mapping" of the major allergens of alder, birch and hazel with respect to the specificities of IgE is currently being undertaken at our laboratory.

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