

Comparison of the *in-vivo* and *in-vitro* response to ragweed immunotherapy in children and adults with ragweed-induced rhinitis

GUNILLA HEDLIN, G. SILBER*, R. NACLERIO*, D. PROUD*, ANA M. LAMAS*, P. EGGLESTON* and N. F. ADKINSON, Jr*

Karolinska Institute Huddinge University Hospital, Huddinge, Sweden, and *Johns Hopkins University School of Medicine, Baltimore, U.S.A.

Summary

In order to compare results of allergen immunotherapy in paediatric and adult populations, 22 children with a history of ragweed hay fever were matched with an equal number of adults for skin sensitivity to ragweed and all were given a 1-year course of immunotherapy with a partially purified ragweed extract. Biological responses were measured by nasal challenges with ragweed before therapy was started, after 12 weekly injections and when the maintenance dose had been reached and also by methacholine bronchoprovocation tests before and after 12 months of therapy. Skin-test sensitivity to ragweed and control allergens, and ragweed-specific IgE and IgG antibody responses were measured at the same intervals as the challenges and at the end of the study. The effect of the therapy on clinical symptoms was not evaluated. Before therapy the groups of adults and children were comparable by all indices, except for TAME esterase activity in nasal washes during ragweed nasal challenge which was significantly lower in children. During treatment, mediators released during sequential nasal challenges declined to undetectable levels in most patients and changes in nasal ragweed sensitivity were comparable in both groups. Ragweed IgE increases after 12 weeks of therapy and IgG levels at maintenance therapy tended to be higher in the children, but neither difference was statistically significant. At the end of the study IgE and IgG antibody levels were comparable in both groups. Results of methacholine inhalation tests did not change significantly in either group. The decrease in skin sensitivity to ragweed was similar in both groups. We conclude that ragweed immunotherapy leads to immunological and biological consequences that are comparable in children and adults.

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Introduction

Immunotherapy has been used for many years as a treatment of allergic rhinitis and asthma in both children and adults. Several studies have suggested that the clinical and immunological effects might be more pronounced in children than in adults. Sadan *et al.* [1] found that, in children receiving ragweed immunotherapy, leucocyte histamine release decreased more strikingly and hay fever symptoms were improved more than had previously been observed in adults. Likewise, Bousquet *et al.* [2] have

recently reported children to improve more than adults after 1 year of dust mite immunotherapy. Nordvall [3] observed that specific IgE increased and rose further after a pollen season in children, contrary to the results found in adults. The more persistent rise in specific IgE in children than in adults has also been reported in a cat and dog immunotherapy study from Sweden which also showed a more vigorous IgG response in the children [4]. The latter finding is in line with the heightened IgG response reported in children treated with venom immunotherapy [5]. Finally, Warner [6] reported that dust mite immunotherapy was more beneficial in asthmatic children than in adults and he hypothesized that children

Correspondence: Gunilla Hedlin, Department of Pediatrics, Huddinge University Hospital, S-14186 Huddinge, Sweden.

might be especially suitable for hyposensitization because 'the treatment could hasten immunological maturation in a favourable way'.

As there are no reported studies which have compared the clinical and immunological effects of immunotherapy in children and adults matched for allergen sensitivity, we undertook the present study. We report here the clinical response to ragweed immunotherapy as assessed by nasal challenges with allergen and methacholine inhalation tests (MIT) in a group of children and adults with histories of rhinitis during the ragweed season. The children and adults were matched for their skin-test sensitivity to ragweed. The effect of immunotherapy on skin sensitivity and ragweed antibody levels was also examined.

Subjects and methods

Subjects

Twenty-two children with a history of rhinitis during the ragweed season were matched for ragweed skin-test sensitivity with the same number of ragweed sensitive adults. Sixteen matched pairs completed the study. The age range for the 16 children was 7–14 yr and 22–40 yr for the adults. Most of the subjects (15 children and 14 adults) also had a history of allergies to other seasonal and/or perennial allergens (Table 1). Eleven of the children and two of the adults had a history of seasonal allergic asthma.

Three children and six adults dropped out before the study was completed. The three children and two of the six adults failed to follow the dose schedule and were far behind when the rest of the group reached maintenance. Two adults dropped out after a few weeks for social reasons. The treatment side-effects, if any, in these seven subjects were very mild; the reason for drop out was lack of compliance. One adult dropped out due to pregnancy and one due to repeated systemic side-effects. Three children remained in the study but without a matched adult and were not included in the data analysis. The protocol was approved by the Johns Hopkins Joint Committee on Clinical Investigation and all subjects were enrolled after giving informed consent.

Study protocol

On the first visit all subjects had puncture skin tests with a panel of seasonal and perennial allergens. On the second visit a MIT was followed by a nasal challenge with ragweed to evaluate ragweed sensitivity. Mediator release (TAME-esterase activity, histamine and PgD₂ concentrations) in nasal washes was measured during the nasal challenges. A skin puncture test titration with ragweed

and a control allergen, not included in the therapy, was also performed. The subjects were matched by ragweed skin-test sensitivity (Table 1). When possible they were also matched according to nasal sensitivity, i.e. the maximum challenge dose given (12 of 16 pairs). On the third visit, blood was drawn for measurement of total IgE and ragweed-specific IgE, IgG and IgG₄. Immunotherapy was then started. Re-evaluation was performed by repeating the nasal challenge after 12 weekly injections (half-way up to maintenance), and again when patients had reached their maintenance dose. The skin-test titration and measurements of ragweed IgE and ragweed IgG were repeated after 12 weekly injections, at maintenance, and at the end of the study. The MIT and screen-test battery were repeated after about 1-year of therapy when the study was completed. The subjects who, due to local or systemic side-effects, did not tolerate the target maintenance dose of 9 µg *Amb a* I were given additional injections of the maximally tolerated dose to reach a cumulative dose as close as possible to their matched pair both at maintenance and at study completion.

Allergen extracts

The ragweed extract was provided by ALK/S (Copenhagen, Denmark) and was a partly purified, well-standardized extract containing 80 µg/ml of the principal allergen, *Amb a* I. A single batch of extract was used for all skin tests, immunotherapy, nasal challenges and specific IgE and IgG measurements *in vitro*.

Other extracts used for skin testing and immunotherapy were also obtained from ALK/S and were partly purified and standardized. The panel included a three-grass mixture of rye, timothy and orchard grass, English plantain, Bermuda grass, white oak, a 50/50 mixture of *Dermatophagoides*, *pteronyssinus* and *D. farinae*, alternaria, cladosporium, aspergillus and cat. The crude tree extracts, white ash, silver and red maple, birch and American elm were obtained from Hollister-Stier (Spokane, U.S.A.) and their potency was expressed in protein nitrogen units (PNU). A dog extract from ALK/S and a cockroach extract from Hollister-Stier were used for skin testing but not for immunotherapy.

Skin tests

Puncture tests with the 17 extracts mentioned above in a diluent with 50% glycerine was performed using a bifurcated Wyeth needle. Skin-test sensitivity to ragweed was quantitated by a titration procedure using five approximately half-log dilutions of ragweed extract (80, 24, 8, 2.4 and 0.8 µg/ml *Amb a* I) in duplicate. The dilution that induced a 3+ reaction (a weal of the same size as that

Table 1. Ragweed sensitivity in adult and children before therapy

Pair no.	Age (yr)/Sex	Skin test* (log µg/ml <i>Amb a I</i>)	Nasal sensitivity		Allergens included in IT	Control allergens	
			Maximum dose (log µg/ml <i>Amb a I</i>)	SS Sn			
Adults							
1	25/M	−0.1	0.90	11	0	PDL, WO, Cat, tree mix	Dog
2	24/F	−0.1	0.90	9	1	PDL, Epl, Dm	Cat
3	34/F	0.38	−0.1	12	13	PDL, Alt, BG	Cat
4	39/F	0.38	−1.1	8	5	—	—
5	35/F	0.38	0.90	12	14	PDL, Dm, Cat, BG	Dog
6	22/F	0.90	0.90	10	8	BG, Dm	Cat
7	33/F	0.90	0.90	9	2	—	—
8	36/F	0.90	−0.1	8	6	PDL, BG, Epl	—
9	28/M	1.38	−0.1	10	11	PDL, Alt, tree mix	Cat
10	27/F	1.38	0.90	4	0	PDL, BG	—
11	32/M	1.90	0.90	20	7	PDL, BG, WO, Dm	Cat
12	39/M	1.90	0.90	1	9	PDL, BG	Cockroach
13	37/M	1.90	0.90	8	0	Alt	Cat
14	30/M	1.90	0.90	2	0	PDL, BG	—
15	40/M	1.90	0.90	7	4	PDL, WO, Dm, Cat	Dog
16	31/M	1.90	−1.1	7	6	PDL, WO, At, Dm	Cockroach
1	9/M	−0.1	−2.1	9	7	—	—
2	10/M	−0.1	−1.1	5	9	BG, PDL	Cockroach
3	13/M	0.38	−0.1	3	6	PDL, Dm	Cat
4	14/F	0.38	−0.1	4	8	PDL, Alt	Cockroach
5	10/M	0.38	0.90	3	0	Dm	Cockroach
6	8/M	0.90	0.90	8	12	PDL, WO,BG	Cockroach
7	7/M	0.90	0.90	6	2	Dm	Cat
8	10/M	0.90	−0.1	3	8	PDL, Epl	Dog
9	11/M	1.38	−0.1	9	8	PDL, Epl, Alt, Dm	Cat
10	7/M	1.38	0.90	10	0	Alt, Dm	—
11	10/M	1.90	0.90	11	0	Cat, tree mix	—
12	14/F	1.90	0.90	3	1	Dm	Cat
13	12/F	1.90	0.90	1	0	Cat	Dog
14	13/M	1.90	0.90	5	0	Tree mix	Cat
15	11/F	1.90	0.90	8	7	—	Alt
16	13/M	1.90	0.90	7	7	Cat, Alt, Dm	Dog

* Concentration of allergen required to produce a skin weal size comparable to that obtained with 10 mg/ml histamine hydrochloride. Immunotherapy (IT), grass-mix (PDL), white oak (WO), English plantain (Ept), dust mite (DM), alternaria (Alt), Bermuda grass (BG). *Amb a I*: formerly ragweed antigen E; SS=symptom score, Sn=number of sneezes.

induced by 10 mg/ml of histamine) was used for the matching of the adults to the children. The log concentration of *Amb a I* in the dilution used for matching in each subject is given in Table 1. When possible a skin-test titration was also performed with a control allergen to which the patient had a positive reaction on the screening test (> 2 mm) but which was not included in the therapy. All skin tests were performed on the volar side of the

forearm as described previously [7]. Histamine hydrochloride (10 mg/ml) was used as a positive control and the glycerine diluent as a negative control. The weal reaction was outlined in pen after 15 min and the outline transferred to graph paper with cello tape. Skin-test results were recorded as the mean weal diameter, calculated by measuring the largest diameter, determining its orthogonal perpendicular and dividing the sum by two.

Nasal challenge

All three challenges were done outside of the ragweed season. The protocol followed has been extensively described before [8]. Briefly, the challenge was started with four pre-washes using instillations of 2.5 ml of normal saline into each nostril while the patient tilted his head 30° from the horizontal in a sitting position. The saline-secretion mixture was recovered and stored on ice. The patient was then given one spray of oxymetazoline hydrochloride (Afrin) nasal spray in each nostril to avoid extensive congestion during the allergen challenge, followed by a wash. The diluent was given thereafter and then 10-fold increasing doses of the ragweed extract (0.00008 to 8 µg/ml *Amb a* I; i.e. up to six challenges doses). The diluent and 0.1 ml of the different concentrations of ragweed extract were instilled into the right nostril from a tuberculin syringe. Ten minutes after the diluent and after each challenge dose a nasal lavage was performed as described above. The wash after diluent challenge was considered the control wash. Samples were centrifuged at $800 \times g$ for 15 min to separate the gel from the sol phase. For histamine determinations 0.8 ml of the sol phase was mixed with 0.2 ml of 8% HClO₄ and stored at -20°C until analysis. For measurements of TAME-esterase activity and PgD₂, solid phase samples were stored at -20°C until analysis.

During the nasal challenge the subjects recorded a symptom score (SS) and the number of sneezes 10 min after each challenge dose. Symptom scores from 1 to 5 were used for itchy, stuffy and runny nose, respectively, and for itchy eyes/throat. A maximum score of 20 could thus be obtained for severe symptoms in response to each challenge dose. The pre-treatment challenge was stopped when the subject reacted with five or more sneezes, or when the highest challenge dose had been given. In either case this was called the maximum dose. Challenges identical to the initial sequence, regardless of the symptomatic response, were performed again after 12 weekly injections and when the maintenance dose was reached.

Mediator assays

The concentration of histamine was determined by an automated fluorometric technique, which is sensitive to approximately 1 ng/ml and accurate to within $\pm 5\%$ [9]. TAME-esterase activity, which is largely due to mast cell tryptase and a plasma kallikrein/alpha macroglobulin complex [10], was determined by the method of Imanari *et al.* [11], which is based on the liberation of tritiated methanol from the synthetic substrate [³H]TAME. The sensitivity of the method was 1000 c.p.m. Prostaglandin D₂ was measured by a competitive radioimmunoassay as

previously described [12]; it is sensitive to 2 pg/0.1 ml. The crossreactivity of the antiserum was less than 1% with PgE₁, PgE₂, TxB₂, 6-keto-PgF_{1α} and PgF_{2α}.

Methacholine challenge tests

Methacholine inhalation challenges were carried out by the method of Chai *et al.* [13]. Aerosols were generated using a DeVilbiss no. 646 nebulizer (DeVilbiss Co., Somerset, U.S.A.), with its auxiliary port open, attached to a French-Rosenthal dosimeter that was breath-activated using a compressed air source (20 p.s.i.). Aerosols were delivered for 0.6 sec at the start of slow inspiration from functional residual capacity to total lung capacity. There was a 1-sec breath hold at the end of inspiration. Nose clips were used during inhalation. Nebulizer output was 20 ± 2 µl (mean $\pm$ s.d.) per breath with a mass median aerodynamic diameter of 1.3 ± 2.6 µm (geometric standard deviation). Five breaths of phosphate-buffered saline (PBS) were inhaled first, followed at 5-min intervals by doubling concentrations of methacholine (Provocholine, Hoffman La-Roche Inc, Nutley, U.S.A.) diluted in PBS to concentrations of 0.075, 0.15, 0.31, 0.62, 1.25, 2.50, 5.0, 10.0 and 25 mg/ml. One millilitre of each solution was added to the nebulizer. Triplicate FEV₁ measurements were made on a Stead-Wells spirometer (Warren Collins; Braintree, U.S.A.) and the best FEV₁ was recorded. Inhalations were continued until the FEV₁ decreased by at least 20%. This drop in FEV₁ was confirmed by a 3 min recording. The result was expressed as PD₂₀, i.e. the dose in cumulative breath units of methacholine that induced a 20% drop in FEV₁.

Ragweed-specific IgE

The ragweed extract was coupled to agarose using a cyanogen bromide method as described previously [14]. The allergosorbent was standardized by reciprocal plot analysis to determine optimal concentrations for use in the antibody assay by RAST. The concentration of IgE was determined by interpolation from a ragweed reference serum curve previously standardized in ng/ml by the RAST elution technique [15]. Quantitation of IgE was undertaken in three dilutions in order to detect and correct for IgG interference according to Adkinson [15]. Results were expressed in ng/ml. Samples from before therapy and after achievement of maintenance treatment were analysed in the same assay. All sample dilutions were analysed in duplicate with an intra-assay coefficient of variance less than 10%.

Ragweed-specific IgG and IgG₄

Samples were analysed for total ragweed IgG using an established *Staphylococcus* protein A solid-phase RIA as

previously described [16]. Ragweed IgG₄ was quantified using a microtitre plate-based indirect ELISA assay employing mouse monoclonal anti-IgG₄ and alkaline phosphatase-conjugated goat anti-mouse IgG [17]. Results were expressed in $\mu\text{g/ml}$ using calibrated reference sera. All samples were analysed in duplicate within the same assay and the intra-assay coefficient of variation for duplicate samples was less than 15%. Assay sensitivity was 0.1 $\mu\text{g/ml}$, corrected to undiluted serum.

Immunotherapy

Immunotherapy was given as weekly injections leading up to a dose of 9 μg *Amb a* I, if possible. In 24 subjects immunotherapy was also given simultaneously with other allergens, chosen according to standard clinical criteria, using data from skin tests, RAST and clinical history. The extracts included in the therapy for individual subjects are shown in Table 1.

Statistics analysis

As data were not normally distributed, non-parametric statistics were used throughout. Wilcoxon's paired sign-rank test was used to compare changes related to therapy. Mann-Whitney U-tests were used to compare children to adults, and Spearman's rank correlation was used to test for association between variables.

Results

Skin tests

All patients had a 3+ or larger weal reaction to ragweed before therapy was started. The distribution of the skin sensitivity is given in Table 1 where the matching of subjects is also illustrated.

The mean weal diameter induced by puncture testing with the undiluted ragweed extract (80 $\mu\text{g/ml}$ *Amb a* I) was comparable in adults (5.6 ± 0.6 mm) and children (4.7 ± 0.6 mm) before therapy. The mean weal size after reaching maintenance and at the end of the study was significantly smaller than before therapy in both groups (3.2 and 2.7 mm) (Fig. 1; $P < 0.01$) and the change was not greater in the children ($P = 0.18$). The mean diameter of the weals induced by the highest concentration of the control allergen did not change in the adults (3.7–3.8 mm), but increased significantly in the children by the end of the study (from 3.5 to 4.8 mm) ($P < 0.05$) (Fig. 1).

The weal sizes of skin tests with other treatment allergens changed to variable degrees. The mean weal size induced by the three-grass mixture did not change significantly in the 18 subjects who were treated with the grass mixture. Neither did the skin sensitivity change in

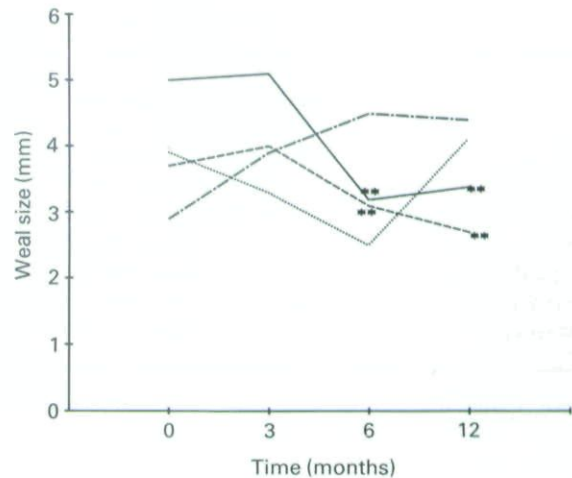


Fig. 1. Median changes in skin sensitivity to ragweed in 16 adults (—) and 16 children (---) during a 1-year course of ragweed immunotherapy, and to a control allergen (not included in the therapy) in 11 adults (· · · ·) and 13 children (- · - ·) expressed as the diameter of the weal reaction (mm) induced by a ragweed extract containing 80 $\mu\text{g/ml}$ *Amb a* I and cockroach or dog extracts. ** $P < 0.01$, difference in comparison to pre-treatment.

those treated with dust mite, cat or English plantain (subjects 13, 7 and 4, respectively). However, there was a significant decrease in the weal sizes induced by alternaria, Bermuda grass and white oak treatment (8, 10 and 4 subjects, respectively) ($P < 0.05$).

Nasal challenge sensitivity pre-therapy

The distribution of maximum doses for nasal provocation in the two groups is illustrated in Table 1. The distribution was similar in both groups; 12 of the 16 pairs were matched for nasal sensitivity expressed as the maximum dose of *Amb a* I given.

Symptom score and sneezes

Mean SS was 9 (± 1) in the adults and 6 (± 1) in the children after the maximum dose before therapy ($P < 0.05$). The number of sneezes did not differ between the two groups (mean approximately 5 in both groups). After maintenance dose was achieved there was no significant difference between the adults and the children with regard to either symptoms scores or sneezes at the maximum dose. Both groups recorded lower SS and fewer sneezes after treatment than before therapy ($P < 0.01$; Table 2). Two subjects (one adult and one child) reported very mild symptoms and did not sneeze. Both, however, had a significant increase in at least one of the mediators measured in nasal secretion and were considered to have recorded a response at the highest dose.

Table 2. Nasal challenge before therapy, after 12 weekly injections and at maintenance

	Before immunotherapy		After 12 weekly injections		Maintenance therapy	
	Children	Adult	Children	Adult	Children	Adult
TAME (c.p.m. $\times 10^{-3}$)	26.6 $\pm$ 6.8 (18.2)	50.6 $\pm$ 7.3 (46.9) [†]	20.0* $\pm$ 10.4 (3.7)	27.3** $\pm$ 8.3 (12.2)	4.0** $\pm$ 2.5 (0)	5.7*** $\pm$ 2.4 (1.4)
Histamine (ng/ml)	2.5 $\pm$ 1.8 (0.2)	3.7 $\pm$ 2.0 (1.0)	1.6 $\pm$ 1.0 (0)	0.70** $\pm$ 0.30 (0)	0.2** $\pm$ 0.1 (0)	0.6** $\pm$ 0.4 (0)
PgD ₂ (pg/0.1 ml)	11.8 $\pm$ 4.1 (7.4)	16.0 $\pm$ 5.9 (7.4)	11.1 $\pm$ 5.5 (4.5)	13.1 $\pm$ 5.6 (0)	6.3 $\pm$ 3.2 (0)	3.8* $\pm$ 2.4 (0)
Symptom scores	6 $\pm$ 1 (6)	9 [†] $\pm$ 1 (9)	3** $\pm$ 0.6 (3)	5** $\pm$ 1 (6)	1** $\pm$ 0.5 (0.5)	3** $\pm$ 0.7 (2)
No. of sneezes	4.7 $\pm$ 1.0 (6.5)	5.4 $\pm$ 0.7 (5.5)	3.7 [†] $\pm$ 0.9 (2.5)	4.6* $\pm$ 1.5 (1)	0.3** $\pm$ 0.2 (0)	1.2** $\pm$ 0.5 (0)

Values represent mean $\pm$ s.e.m. for maximum challenge dose, with medians in parentheses.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, difference in comparison therapy.

[†] $P < 0.05$, difference between children and adults.

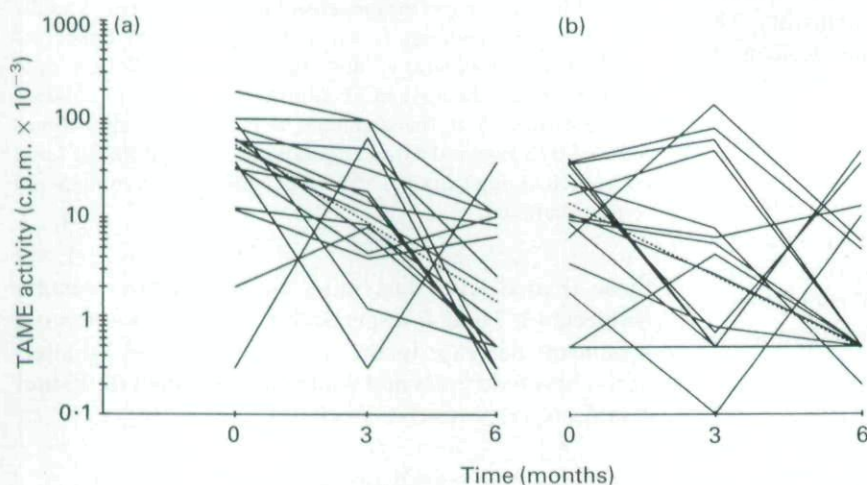


Fig. 2. TAME-esterase activity in 16 adults (a) and 16 children (b) at the maximum dose in the nasal challenge before therapy and at the identical dose of nasal challenges after 12 weekly injections and when the maintenance level of treatment had been reached. Median values are indicated.

Mediator release

TAME-esterase activity at the maximum challenge dose was significantly higher in the adults than in the children before therapy ($P < 0.05$), but almost identical in the two groups at the maintenance level (Table 2). The decrease after therapy was significant in both groups ($P < 0.001$ and $P < 0.01$). Before therapy 14 of the 16 children and all of the adults were TAME responders, defined as achieving more than a twofold increase in TAME activity over that in the control wash. After reaching maintenance, five children and eight adults still had a TAME response. The individual TAME responses are illustrated in Fig. 2.

Increased histamine in the nasal secretion (> 1 ng/ml above the control wash) was found in eight children (50%) and 12 adults (75%) before therapy and in two children (13%) and three adults (19%) at maintenance (Table 2). The decrease in the histamine elevations after treatment was significant for both groups ($P < 0.01$). There was no significant difference between adults and children either before or during therapy.

Prostaglandin D₂ could be measured during antigen challenge in 11 children (69%) and in seven adults (44%) before therapy. After therapy there remained five children (31%) and four adults (25%) who still had measurable increases in PgD₂ levels. The mean PgD₂ concentration decreased in both the adults and the children (Table 3; $P < 0.05$ and $P = 0.06$). There was no significant difference in the mean PgD₂ levels between children and adults at any time during the study (Table 2).

Antibody measurements

Mean total serum IgE measured before therapy was higher in the children (1582 ng/ml) than in the adults (715 ng/ml), ($P < 0.05$; Table 3).

Ragweed-specific IgE levels were comparable between children and adults before therapy (Table 3). After 12 weekly injections, ragweed IgE increased significantly in both groups. By the time maintenance level treatment had been achieved, ragweed IgE had declined in both groups and mean values were not different from pre-treatment

Table 3. Antibody levels and cumulative dose of *Amb a 1* over the course of immunotherapy in matched adult-child pairs

Variable	Before immunotherapy		After 12 weekly injections		At maintenance therapy		At end of study	
	Children	Adults	Children	Adults	Children	Adults	Children	Adults
Total IgE (ng/ml)	1582 ± 377 (1259)	715 ± 199† (304)	—	—	—	—	—	—
Ragweed IgE (ng/ml)	143 ± 56 (20)	105 ± 40 (23)	232 ± 82*** (78)	180 ± 75** (37)	178 ± 68 (40.0)	127 ± 50 (36)	93 ± 38*‡ (30)	79 ± 46*‡ (21)
Ragweed IgE (µg/ml)	0.21 ± 0.13 (0)	0.11 ± 0.06 (0)	0.43 ± 0.15** (0.23)	0.32 ± 0.12** (0.19)	4.8 ± 0.9*** (4.0)	3.8 ± 0.6*** (2.9)	3.3 ± 0.6*** (2.8)	2.8 ± 0.4*** (2.4)
Ragweed IgG ₄ (µg/ml)	0	0.05	—	—	0.26 ± 0.11** (0.06)	0.63 ± 0.25** (0.13)	1.05 ± 0.36*** (0.42)	0.83 ± 0.27*** (0.43)
Cumulative dose of <i>Amb a 1</i> (µg) (range)	0	0	0.11	0.11	60.0 ± 4.5 (12–81)	56.3 ± 4.2 (11–79)	123 ± 10 (23–190)	131 ± 11 (19–177)

Values represent mean ± s.e.m., with medians with parentheses.

Ragweed IgG₄ was determined in 10 children and 11 adults.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, difference in comparison to before therapy.

† $P < 0.05$, difference between children and adults.

‡ $P < 0.05$, difference between maintenance and end of study.

levels. By the end of the study, serum ragweed IgE had decreased further in both groups and was then significantly lower than at the start ($P < 0.05$ for both; Table 3).

Ragweed-specific IgG levels were detectable ($> 0.1 \mu\text{g/ml}$) in five children and in three adults before therapy. After the initial 12 weekly injections nine children and an equal number of adults had IgG levels $> 0.1 \mu\text{g/ml}$ ($P < 0.01$). At maintenance all children and adults had IgG levels higher than $1.5 \mu\text{g/ml}$ (mean $4.8 \mu\text{g/ml}$ for children and $3.8 \mu\text{g/ml}$ for adults; $P > 0.10$). At the end of the study IgG levels had decreased somewhat in both groups ($3.3 \mu\text{g/ml}$ and $2.8 \mu\text{g/ml}$, respectively), but the groups still did not differ significantly (Table 3). Ragweed-specific IgG₄ levels were not detectable ($< 55 \text{ ng/ml}$) in any of 10 children for whom complete sets of serum were available. At maintenance, four of seven measurements showed IgG₄ levels $> 55 \text{ ng/ml}$ and at the end of the study all 10 children had ragweed IgG₄ ranging from 69 to 3227 ng/ml (Table 3). In the adults ragweed IgG₄ was measured in 11 subjects who had complete serum sets. Two subjects had detectable IgG₄ before the study, all 11 at maintenance (range 58–2219 ng/ml). By the end of the study ragweed IgG₄ ranged from 273 to 3318 ng/ml (Table 3). There were no significant differences between children and adults at any time. The correlations between ragweed IgG and IgG₄ were strong ($r = 0.96$ and 0.95 at maintenance in children and adults, respectively; and $r = 0.92$ and 0.89 at the end of the study).

Intercorrelations

There was a weakly predictive but significant correlation between the change in the number of sneezes and the

change in the TAME-esterase activity in the whole group ($r = 0.32$; $P < 0.05$). The decrease in TAME-esterase activity also correlated significantly with the change in the histamine concentration in the whole group ($r = 0.45$; $P < 0.01$). The decrease in the PgD₂ levels did not correlate significantly to the change in TAME-activity ($r = 0.30$; $P = 0.05$), but was correlated to the decreased histamine concentration ($r = 0.37$; $P < 0.05$). No correlation was found between the decrease of SS, number of sneezes, or any mediator released and changes in skin sensitivity or ragweed IgE. In the children ragweed IgG at maintenance and at the end of the study correlated with the change in TAME-esterase activity ($r = 0.46$; $P < 0.05$ and $r = 0.63$; $P < 0.01$). This correlation was not significant among adults ($r = 0.02$ and $r = 0.20$). Ragweed IgG₄ also correlated with the decrease in TAME-esterase activity in the children ($r = 0.58$; $P < 0.05$ and $r = 0.59$; $P < 0.05$) but these correlations were also not significant in the adults ($r = 0.37$ and $r = 0.36$).

Methacholine challenge results

Twenty-two subjects (seven adults and 15 children) had a fall in FEV₁ of more than 20% during the first and second challenge. There was no significant change in PD₂₀ in the 'methacholine responders'; mean PD₂₀ was 10.6 BU before and 11.8 BU after 1 year of treatment. Two subjects required more than one doubling concentration to react on the second challenge. The remaining 20 subjects reacted within one doubling concentration of methacholine after 1 year of treatment.

Ten subjects completed the first MIT without evidence of bronchial hyperreactivity before therapy; none of them had a history of asthma. After 1 year of therapy nine of

these subjects still had a negative MIT while one had a 20% decrease in FEV₁ after 10 mg/ml of methacholine had been administered.

Treatment course and reactions to immunotherapy

The mean cumulative dose of *Amb a* I at maintenance was comparable in adults (56.3 µg; range 10.8–79.1 µg) and children (60.0 µg; range 12.1–81.1 µg). The number of weekly injections required to reach maintenance ranged from 28 to 37 in the adults and from 29 to 38 in the children. At the end of the study the mean cumulative dose in adults was 131 µg (range 19–188 µg) and in children 124 µg (range 23–190 µg). The individual cumulative doses are presented in Table 1.

Side-effects of therapy

Systemic side-effects occurred in six adults and in two children. Most of them were mild (itchy throat or general pruritus) and subsided after one dose of antihistamine. One child had one episode and one adult had four episodes of general urticaria which had to be treated with injections of antihistamine and epinephrine. No life-threatening side-effects occurred. Large local reactions (induration > 3 cm) were observed in five children and five adults during the course of therapy.

Discussion

Children are widely believed to outgrow their allergies, and particularly asthmatic symptoms, as they approach adulthood [18,19]. Hay fever, however, has a tendency to persist and a relatively low remission rate in patients with rhinitis has been reported [19]. In the current study a majority of the children gave a history of asthmatic symptoms as well as rhinitis while only two adults had asthma. This observation might have been caused by a biased selection of patients, but it could also reflect the spontaneous improvement of asthmatic symptoms with age.

This study provided the possibility of comparing both *in-vivo* and *in-vitro* effects of immunotherapy in children and adults with ragweed-induced rhinitis. Previous immunotherapy studies including both children and adults were not designed to provide comparisons of equally sensitive paediatric and adult populations. The aim of the present study was therefore to evaluate the response to ragweed immunotherapy in children and adults matched for ragweed sensitivity.

We observed a difference in symptom scores and TAME-esterase activity during nasal antigen provocation before therapy was started. Both indices were higher

in adults than in children. One could speculate that in line with the observations of Rawle *et al.* [20], there was an age-dependent decrease in skin sensitivity to allergen. Thus, skin reactions of equal sizes could be associated with more severe rhinitis in the adults than in the children. Another possibility is that skin-test sensitivity in the children reflected more the sensitivity in the lower airways than in the upper airways as so many children had both seasonal asthma and rhinitis.

A good concordance between allergen-induced sneezing and the three nasal mediators has been reported by us previously [7]. We have also reported significant, although weak, correlations between changes in histamine or PgD₂ in nasal washes and changes in symptom score during challenge with cold dry air [21]. TAME-esterase activity, however, did not have a significant correlation to symptom score in that study. In the present study we found the same weak correlations between changes in mediator release and the decreased number of sneezes. One tentative explanation for the weak correlations could be the insensitivity of the lavage technique to low levels of mediator release, as shown by the inability to measure mediators in some nasal lavage samples of clinically positive challenges.

TAME-esterase activity and histamine levels measured in recovered lavages during nasal allergen provocation decreased significantly after immunotherapy. TAME-esterase activity decreased less in the children than in the adults which could be due to the initially lower TAME activity in children. PgD₂ levels decreased slightly more in the adults than in the children. The PgD₂ change in the children would probably have been significant had there been more patients in the study. Thus, a comparable decrease in mediators in both groups indicates a similar effect in response to treatment. This effect of the therapy on target organ sensitivity is in agreement with the result of a previous study of cat immunotherapy [22] in which children and adults were reported to have comparable effects of the therapy on bronchial allergen sensitivity. The children and adults who took part in that study were, however, not matched for allergen sensitivity when started on therapy.

Skin sensitivity to ragweed decreased as reported in other pollen and animal dander immunotherapy studies [23–25], and was similar in adults and children.

The change in skin sensitivity to the control allergen in the children was found only when the initial skin test was compared to the last skin test. If the skin test made after 12 weeks of therapy was compared to the last there was no change. We therefore assume that the change in the control skin test is spurious and caused by normal variation.

The effect of the therapy on serum ragweed antibodies

was comparable in both groups. A tendency towards higher IgG production in children was observed, but the difference was not statistically significant. In our previous report on mediator release during early immunotherapy, we failed to find a quantitative relationship between the effect of the therapy on the antibody levels (IgG and IgE) and the effect on the mediator release during nasal challenge [26]. In another recent study from our clinic, Creticos *et al.* [27] found no significant correlations between the effect of immunotherapy on nasal mediator release or skin sensitivity and the ragweed a specific IgG response. The correlation we found in this study between the decline in TAME-esterase activity and the increase in ragweed IgG and ragweed IgG₄ in the children could reflect a causal relationship between clinical effect and antibody-production. However, we doubt this to be the case; first because the same pattern was not seen for histamine, and secondly, because the correlation was not observed in the adult patients. We thus believe these significant correlations are likely to be spurious with respect to their biological significance.

The result of the methacholine challenge before therapy confirmed bronchial hyperreactivity (BHR) in all subjects with histories of asthma. In addition, eight subjects (three children and five adults) without asthma were shown to have hyperreactive airways. Immunotherapy did not change hyperreactivity significantly in any of the patients and none of those with a negative MIT developed BHR during the therapy. Furthermore, there was no indication that 1 year of multiple allergen treatment had any beneficial effect on BHR. Our finding is in agreement with two reports of cat immunotherapy-treated patients [25,28] and the recent study of effects of dust-mite immunotherapy in children and adults by Bousquet [1]. A Swedish study of cat immunotherapy has, however, shown BHR measured by histamine broncho-provocation tests to decrease continuously during a 3-year course of therapy [22]. In another Swedish study, BHR increased less during the birch pollen season in an immunotherapy-treated group than in a placebo-treated group [29], while in contrast, a Canadian study of dust-mite treated children has shown a tendency toward increased BHR in treated patients compared to a control group [30]. It is thus still doubtful whether immunotherapy affects BHR. It might be that continued natural exposure to the allergen, as with dust mite, attenuates treatment effects on BHR, and persistent treatment together with allergen avoidance will be necessary to achieve a major effect on BHR.

In conclusion, there was a tendency towards a more vigorous antibody response in the children, but overall we failed to show significant differences between adults and children. The hypothesis about a more pronounced *in-*

vivo and *in-vitro* response to immunotherapy in children could not be confirmed. However, because of drop outs, the final number of matched pairs was small. The possibility remains that the differences in the antibody response between the two groups would have been statistically significant had there been larger sample sizes.

An important question that remains to be answered is whether immunotherapy during childhood alters the future course of the disease. Johnstone [31] found that there was a higher remission rate in children with asthma undergoing immunotherapy than in those who were given only pharmacological treatment. To our knowledge, subsequent studies of the influence of immunotherapy on remission rates have not been undertaken, so that this important potential advantage of immunotherapy in children has yet to be confirmed.

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