

Allergen-specific IgE and IgG antibodies in pollen-allergic children given immunotherapy for 2–6 years

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Summary

Ninety-five children with pollen hay fever were treated for 46 months (16–74 months) with extracts of birch pollen (seventy-two children) and/or grass pollen (fifty-nine children).

During the first 2 months of this immunotherapy the serum concentration of allergen-specific IgE antibodies doubled or tripled, but the concentration of IgG 'blocking' antibodies increased even more.

With time the IgE antibody activity decreased in 80% of the children, but only in slightly more than 50% did it fall to below the initial level, and the mean concentration at the end of the study was 2–3 times the initial value. The IgG antibody activity remained high in about 50% of the children and showed a tendency to decrease in the other children, in spite of monthly allergen injections.

Eighty-three out of the ninety-five children reported a moderate to marked improvement. At the end of the study the eighteen almost symptom-free children had a higher IgG/IgE antibody ratio than the twelve children who had not improved, but this difference was not significant.

After treatment, thirteen children tolerated ten to 10,000 times more allergen than before by naso-conjunctival challenge tests. Ten of the children reacted to the same allergen dilution as before and two reacted to a ten times weaker extract. Clinical improvement was accompanied by increased tolerance in most patients.

Introduction

Most allergists agree that the patient with pollen allergy is improved but seldom cured by immunotherapy (Lichtenstein, 1972; Sørensen, 1976). The mechanisms underlying this beneficial effect are still obscure. The increase in reaginic activity in the serum during the early phase of immunotherapy has been well established by biological and

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immunological techniques (Connell & Sherman, 1969; Levy & Osler, 1967; Berg & Johansson, 1971; Lichtenstein *et al.*, 1973). In spite of this, the patient does not seem to become more sensitive, which has been attributed to the simultaneous occurrence of allergen-specific non-reaginic antibodies competing for allergen with the IgE (reaginic) antibodies bound to the mast cells and basophils. The competitive inhibition by 'blocking antibodies' of the binding of allergen to cells which stimulate immuno-competent B lymphocytes to mature to IgE antibody-producing cells is regarded as a possible explanation for the decreasing reaginic activity seen with time in patients undergoing immunotherapy (Irons, Pruzansky & Patterson, 1975).

Our knowledge of the long-term effect of immunotherapy on the concentration of antibodies belonging to the reaginic and non-reaginic classes of immunoglobulins is still limited, and the results are to some extent contradictory. Some research groups have found that the characteristic increase of the pollen-specific IgE antibody concentration during the pollen season does not develop in patients undergoing immunotherapy (Sadan *et al.*, 1969; Levy *et al.*, 1971; Reisman, Wypych & Arbesman, 1975), but this has not been confirmed in other reports (Yunginger & Gleich, 1973; Foucard & Johansson, 1976). The percentage of patients with decreasing IgE antibody titres during therapy varies from about 50% to almost 100% (Sherman, Stull & Cooke, 1940; Connell & Sherman, 1969; Lichtenstein *et al.*, 1973). The concentration of 'blocking' (IgG) antibody titres remains high or decreases with time (Sherman *et al.*, 1940; Sadan *et al.*, 1969; Melam *et al.*, 1971). The discrepancies between the reported results may reflect differences in the composition of the patient materials and in the schedules used for immunotherapy. Most studies have been performed on a small number of patients who have probably been treated more intensively than the usual patient in the clinic. As the clinical improvement is positively correlated to the amount of allergen given (Johnstone & Dutton, 1968; Starr & Weinstock, 1970; Melam *et al.*, 1971; Lichtenstein *et al.*, 1973), the aim of the present study was to investigate the changes in allergen-specific IgE and IgG antibody concentration in relation to the clinical course in a large number of patients given immunotherapy according to the routine schedule used in our clinic.

Materials and methods

Ninety-five children suffering from allergic rhino-conjunctivitis, with or without bronchial asthma during the tree pollen and/or grass pollen season, were examined. All patients were skin prick test-positive and RAST-positive to a birch pollen and/or a Timothy pollen extract. Seventy-two children received immunotherapy with birch pollen extracts and fifty-nine with grass pollen extracts. Thirty-six of the children were treated with both extracts. Most children were treated simultaneously with other allergens such as extracts of other pollens, house dust, animal danders or moulds.

The immunotherapy was in the form of aqueous extracts (seventy-four children) or alum-adsorbed (Allpyral) depot preparations (twenty-one children). The treatment schedule was identical with that used routinely in our allergy clinic. The aqueous extract was given as a rush hyposensitization (Berg & Johansson, 1971) with 1.0 ml of a 10^{-2} w/v dilution as the highest maintenance dose of allergen, which was given monthly after the induction period. Local or general reactions often necessitated lower maintenance doses, but the aim was to increase the dose at each visit. The Allpyral extract was given according to the recommendations by the manufacturer, with weekly intervals during the early phase and with intervals of 5–6 weeks between the

maintenance doses, which never exceeded 1.0 ml of a 10,000 PNU/ml extract. The mean total dose given to the patients treated with Allpyral extracts was 160,000 PNU. During the respective pollen seasons the doses of both extracts were reduced to 1/4–1/5 of the maintenance doses.

The mean duration of treatment was 46.3 months (range 16–74 months). The patients receiving aqueous extracts were treated, on an average, for 48 months, as compared with 40 months for those receiving Allpyral. No symptom scores were recorded and the clinical effectiveness of the treatment was judged from the reports by the patients and their parents regarding symptoms during the pollen seasons of the treatment period as compared with previous years.

Provocation tests

Naso-conjunctival provocation tests were performed as end-point titrations, using ten-fold dilution steps as described earlier (Berg, Bennich & Johansson, 1971).

Blood samples

Venous blood samples were taken from all patients before and after the treatment period, and from most patients one or more samples were also taken during this period. The mean number of blood samples was 5.1 per patient. After clotting, the serum was withdrawn and kept frozen until analysed.

Allergens

Aqueous extracts, 1/10 w/v, of birch tree pollen and Timothy grass pollen (Vitrum Laboratories, Stockholm, Sweden) were used for skin prick tests and in RAST. Dilutions of the same extracts were used for provocation tests and immunotherapy. Allpyral three-tree mix and five-grass mix (Dome Laboratories, New York, distributed by MEDA Ltd, Gothenburg, Sweden) were used for immunotherapy only. In the assay for blocking antibodies, preparations of Timothy grass pollen (Mullan, 1974) (kindly supplied by Mr B.G. Overell, Bencard, London) and partially purified birch pollen (kindly supplied by Mr H. Schröder, Pharmacia, Uppsala) were used.

Total IgE

The serum IgE concentration was assayed by Phadebas® PRIST (Johansson, Berglund & Kjellman, 1976).

Allergen-specific IgE antibodies

These were quantified by the radioallergosorbent test (RAST), essentially as described (Johansson, Bennich & Berg, 1971), but using paper discs as the solid phase.

Allergen-specific IgG antibodies

These were measured by a sandwich technique using a Sepharose–protein A complex for adsorption of IgG and ¹²⁵I-labelled allergen preparations, as described in detail for Timothy pollen allergen (Foucard & Johansson, 1976).

Results

Changes in antibody concentrations

During the first week of treatment no changes in the antibody titres were observed

Table 1. Timothy pollen antibody levels as percentages of pre-treatment values during immunotherapy

Duration of treatment	Number of patients	IgG antibody (percentage of initial level)	IgE antibody (percentage of initial level)
Less than 1 week	7	97	103
1-8 weeks	25	554	203
16-74 months	59	5414	287

Table 2. Birch pollen antibody and total IgE levels as percentages of pre-treatment values during immunotherapy

Duration of treatment	Number of patients	IgG antibody (percentage of initial level)	IgE antibody (percentage of initial level)	Total IgE (percentage of initial level)
Less than 1 week	9	107	102	94
1-8 weeks	24	360	290	152
16-74 months	72	354	228	134

(Tables 1 and 2). After 1-8 weeks of therapy the concentration of allergen-specific IgG ('blocking') antibodies had increased in 63% of the children treated with the grass pollen extract (T) and in 74% of those treated with the birch pollen extract (B). The mean titres were 554% (T) and 360% (B) of the initial values, respectively (Tables 1 and 2). A similar trend was found for the allergen-specific IgE antibodies. An increased titre was found in 60% (T) and 71% (B) of the children, with mean values of 203% and 290% of the initial values respectively. With time the antibody titres decreased. This was most evident for the IgE antibodies, which had decreased in 76% (T) and 88% (B) of the children at the end of the study. However, the titres had fallen below the initial level in only 51% (T) and 67% (B) and the mean titres were still two to three times higher than the initial ones (Tables 1 and 2). The concentration of IgG 'blocking' antibodies also showed a tendency to decrease with time. Such a reduction was found in 33% (T) and 65% (B) of the patients, but the titres decreased below the initial level in only five out of the fifty-nine grass pollen-sensitive and in sixteen out of the seventy-two birch pollen-sensitive patients. In all but two of these patients, the IgE antibody concentrations had also fallen below the initial level. The changes of the serum IgE concentrations were less obvious, but were parallel with those of the allergen-specific IgE antibodies (Table 2).

Usually the RAST response was similar to both allergens in children treated with both the grass pollen and the birch pollen extract. Thus in 78% of these children the two allergens caused parallel increases or decreases in the titres.

Aqueous vs Allpyral extracts

In comparison with the children treated with the aqueous Timothy grass pollen extract, those given Allpyral showed a significantly greater increase in the concentration of

Table 3. Timothy and birch pollen antibody levels as percentages of pre-treatment values and IgG/IgE antibody ratios in children treated for 2–6 years with aqueous or alum-adsorbed allergen extracts

Extract		Number of patients	IgG antibody (percentage of initial level)	IgE antibody (percentage of initial level)	IgG/IgE antibody ratio
Timothy	Aqueous	47	2274	223	49
	Allpyral	12	17714	536	1289
Birch	Aqueous	57	362	233	91
	Allpyral	15	325	208	40

allergen-specific IgG antibodies ($P < 0.001$), allergen-specific IgE antibodies ($P < 0.01$) and in the IgG/IgE antibody ratio ($P < 0.05$). On the other hand, the aqueous and the Allpyral extracts gave similar changes in the antibody concentrations in the children treated for birch pollen allergy (Table 3).

Duration of treatment in relation to clinical course

No significant differences were found in the changes in the antibody concentrations or in the IgG/IgE antibody ratio between the eight children treated for less than 3 years (mean 26 months) and the eleven children treated for more than 5 years (mean 65 months). But ten out of the eleven children treated for more than 5 years reported clinical improvement, as compared with five out of the eight children treated for less than 3 years. Four patients treated unsuccessfully for 3 years were given intensive treatment, with injections every week or fortnight during the half-year preceding the next pollen season. The concentration of blocking antibodies was doubled or tripled during that period, while the RAST levels decreased somewhat. However, no definite clinical improvement occurred.

Antibody titres in relation to clinical course

At the end of the treatment period, thirty-three children reported no, or very slight, allergic symptoms during the pollen season, fifty reported a moderate improvement and twelve claimed that their symptoms were unchanged. The eighteen patients who had become almost free from symptoms tended to have higher IgG blocking antibody levels, lower IgE antibody levels and a higher IgG/IgE antibody ratio than the twelve patients who did not improve, but these differences were not statistically significant. A similar, but less marked, trend was found in the children allergic to birch pollen (Table 4). The higher birch RAST level in the successfully treated group was due to one patient with a very definite increase. Omitting this patient, the RAST levels would have been very similar in the two groups.

No correlation was found between a rising serum IgE concentration during the first weeks of treatment, as a sign of an early immune response, and a good clinical effect.

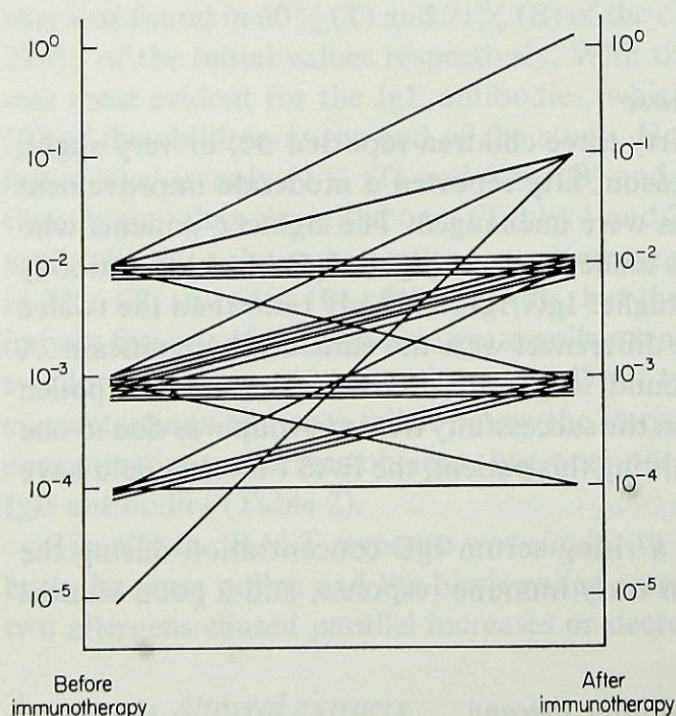
Naso-conjunctival provocation before and after therapy

Twenty-four children, ten (B) and fourteen (T), including one treated with both extracts, were challenged before and after the treatment period. The same batch of

Table 4. Allergen-specific IgG and IgE antibody levels as percentages of pre-treatment values, and IgG/IgE antibody ratios, in unsuccessfully and successfully treated children

Extract	Result of treatment	Number of patients	IgG antibody (percentage of initial level)	IgE antibody (percentage of initial level)	IgG/IgE antibody ratio
Timothy	—	8	838	325	27
	+	10	4312	150	36
Birch	—	10	207	95	13
	+	15	364	165	132

Timothy grass pollen extract was used before and after therapy in nine patients. The stock solution had been stored at 4°C during the treatment period and fresh dilutions were used. In the other patients, extracts considered to be equipotent were used on the two test occasions. After treatment the tolerance had increased by one or more ten-fold titre steps in thirteen children and was unchanged in ten children (Fig. 1). Two children reacted to a grass pollen extract ten times more diluted than before the treatment period, although they claimed to have improved slightly during the treatment. Most patients with increased tolerance to allergen at the provocation tests reported a marked clinical improvement during the season, but there were exceptions. Some patients who had become almost free from symptoms reacted to the same allergen concentration as before the treatment period, and one patient who tolerated a 100 times more concentrated grass pollen extract after therapy claimed only slight clinical improvement.

**Fig. 1.** Titre at nasoconjunctival provocation before and after immunotherapy. Increased tolerance, 13; unchanged, 10; decreased tolerance, 2.

Discussion

A large number of double blind studies have documented the clinical benefits of immunotherapy in patients with pollen allergy (for review see Sørensen, 1976). Although no placebo-treated controls were used in our investigation, the clinical efficacy based on the patients' reports was similar to that observed in other studies (Sørensen, 1976).

The increasing concentrations of serum IgE and allergen-specific IgE and IgG antibodies during the early phase of the treatment are in accordance with other reports (Connell & Sherman, 1969; Sadan *et al.*, 1969; Berg & Johansson, 1971; Melam *et al.*, 1971; Yunginger & Gleich, 1973). The true increase in the IgE antibody concentration was probably even greater, as the presence of blocking antibodies interferes with the binding of the IgE antibody to the solid phase allergen in the RAST to some extent (Wide, Aronsson & Wiholm, 1975). On the other hand, the figures for the blocking antibody titres are probably too high, as they include some IgE antibody activity to non-allergenic protein in the allergen extract.

After 2–6 years of treatment, the IgE antibody titres had decreased below the initial level in 51 to 67% of the patients. These figures are very similar to those reported by Connell & Sherman (1969), who found a significant decrease in the skin-sensitizing antibody titre in ten out of twenty-one patients treated for ragweed allergy for 1–3 years. In an older study, Sherman *et al.* (1940) reported a striking reduction in the skin-sensitizing antibody activity in twenty-five out of twenty-nine sera from patients treated for 10–23 years. Seven of these sera no longer passively sensitized human skin. A more rapid decrease in the IgE antibody activity was reported by Levy *et al.* (1971), who found decreasing activity in eighteen out of nineteen patients after only 15 months of therapy. On the other hand, Melam *et al.* (1971) noted a decreasing skin-sensitizing antibody titre in only two out of seven patients after 1 year of treatment. The two patients with decreasing titres had both received ten to thirty times more allergen than the other patients. This relationship between the amount of allergen given and the cellular responsiveness, the blocking antibody titres and the clinical response has been emphasized in several studies (Sadan *et al.*, 1969; Starr & Weinstock, 1970; Levy *et al.*, 1971; Melam *et al.*, 1971; May *et al.*, 1972; Lichtenstein *et al.*, 1973). The comparatively small difference in the total amount of allergen given to patients treated for short and long periods in our study may explain the lack of significant differences in the antibody concentration changes between the two groups. Nor was there a significant correlation between the reported clinical improvement and the titre of blocking antibody, the titre of IgE antibody or the IgG/IgE antibody ratio. But the twelve children with no clinical improvement after immunotherapy had a smaller increase in the IgG antibody titre, a lower IgG/IgE antibody ratio and a greater increase in the IgE antibody titre than the eighteen patients with excellent clinical improvement, indicating that these parameters do reflect the clinical response to some extent. Four patients with no clinical improvement after 3 years of therapy already had high IgG antibody titres before treatment, and responded with a very modest increase in their titres during therapy. They were given intensive treatment for several months preceding the following pollen season. In all four patients the blocking antibody titres increased and the IgE antibody titres, which had started to decrease earlier, showed a further reduction during the period of intensive therapy. In spite of this, no great clinical improvement was reported by the patients, which discouraged them from further immunotherapy. The possible increased benefit of a more intensive therapy

schedule must be considered against the inconvenience of more frequent visits to the clinic and probably an increased risk of local or systemic reactions.

The Timothy grass pollen extract seemed to stimulate the production of blocking antibodies better than the birch pollen extract. This was further substantiated by the finding that during the maintenance therapy, decreasing IgG antibody titres were found in 65% of the children treated with the birch pollen extract, as compared with 33% of the children treated with the extract of Timothy grass pollen.

No significant difference was found between the changes in antibody concentration induced by aqueous *vs* Allpyral birch pollen extracts. The Allpyral grass pollen extract was found to give significantly higher concentrations of blocking antibodies than the aqueous extract, but it also induced a significantly higher IgE antibody concentration. As this increase was less evident than that of the IgG antibodies, the IgG/IgE antibody ratio was much in favour of Allpyral. As the pollen depot preparations seemed to be at least as effective clinically as the aqueous extracts, required fewer injections and gave fewer side reactions, we now use Allpyral extracts in most of our patients with pollen allergy.

The lack of a convincing correlation between the changes in the allergen sensitivity thresholds and the reported degree of clinical improvement during the pollen season surprised us. Although part of this discrepancy may have been due to methodological errors and heterogeneity of the allergen extracts used before and after therapy, other mechanisms are probably involved. How should one explain that patients who had had severe symptoms during the pollen season before treatment and had become almost free from symptoms during treatment reacted to the same concentration of the allergen extracts on the two test occasions? Eight patients who tolerated ten times more allergen after treatment and were clinically improved still reacted to a 10^{-2} or 10^{-3} w/v dilution of the allergen extract. Such dilutions are often found to be the end-point titre in untreated patients with severe allergic symptoms during the pollen season. The findings indicate that the naso-conjunctival provocation test is a very blunt instrument for estimating the severity of the allergy. The reason for this may be that a provocation test is characterized by the application of a fairly large amount of allergen to the shock organ within a short period of time, whereas during the pollen season the patient is exposed to smaller amounts over a longer period of time.

The allergen-IgE reaction at the mast cell or basophil level is considered to be just one facet of inflammation, and the mediators released from the triggered target cells will secondarily activate other cells and mediator systems which will probably contribute considerably to the clinical symptoms (Austen, Wasserman & Goetzl, 1976, Lichtenstein, 1976). These secondary effects are not fully evaluated in the provocation test. Further evidence supporting this hypothesis is the excellent clinical effect of corticosteroids on subjective hay fever symptoms, even though these drugs are considered to cause no or very little inhibition of the type I reaction (Pepys, 1973). It is thus possible that the effect of hyposensitization is complex, involving blocking antibodies, mechanisms inducing a decreased IgE antibody production and hitherto unknown inhibitory effects on one or more of the biochemical amplifier systems involved in allergic inflammation. This would explain the relative lack of correlation obtained when only one of these parameters is compared with the clinical efficacy of immunotherapy.

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