

Predictive value of serum IgE levels during rush hyposensitization

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Summary

Thirty children with hay fever were studied with *in vivo* and *in vitro* tests during rush hyposensitization with Timothy pollen. Clinical assessment was made with the aid of diaries during one season before, and three seasons after, the start of the immunotherapy. When the serum level of IgE increased more than 10% during the first week of the therapy, symptoms were mostly alleviated during the following seasons. An alleviation was rarely found in children with decreasing or unchanged serum IgE levels. No predictive information as to the clinical effect of the immunotherapy was obtained from skin or provocation tests, nor from Timothy-specific antibodies of the IgG or IgE classes. It is proposed that serum IgE determinations should be performed before, and 1 week after, the start of rush hyposensitization in order to find probable therapeutic failures.

Introduction

Hyposensitization (HS) through the injection of increasing doses of allergen was introduced in the therapy of hay fever some 65 years ago (Noon, 1911; Freeman, 1911). Controlled studies have shown that HS in most cases of hay fever alleviates symptoms and also prevents the development of new allergies (Lowell & Franklin, 1963; Johnstone, 1975). The 'rush' HS, i.e. the injection of allergen every second hour during approximately 5–7 days, has been used since 1930 (Freeman, 1930; Berg & Johansson, 1969).

Reaginic activity increases in the serum during the first 3 months of specific hyposensitizing therapy (Sherman, 1941), but during prolonged therapy such reagins seem to decrease. Total serum IgE levels increase during the first 3–4 days of a rush HS (Berg & Johansson, 1969). However, individual patients seem to react in different ways. An even more pronounced initial increase was found for specific IgE antibodies, as determined with the radioimmunoassay test (Phadebas® RAST) (Berg & Johansson, 1971). The production of so-called blocking antibodies (Cooke *et al.*, 1935), most of which seem to belong to the IgG class (Lichtenstein *et al.*, 1973), is said to be important for the clinical effects of HS.

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Different *in vivo* and *in vitro* methods have been tried for assessing the efficiency of HS treatment (Noon, 1911; Freeman, 1930; Sherman, 1941; Levy *et al.*, 1971). The clinical data should always be taken into account, which is best done by comparing detailed symptom scores before and after HS (Levy *et al.*, 1971). Such an assessment can only be made one season or more after the start of the treatment and is, of course, subject to doubts as to its reliability (Pepys, 1975). A simple and reliable method for the early prediction of the efficiency of HS treatment has not been described. Hence it was of interest to evaluate the predictive significance of findings in case histories, as well as in *in vitro* and *in vivo* tests, before and during the first month of rush HS.

Materials and methods

Patients

Thirty children, 9.1 ± 2.5 years old (mean $\pm$ s.d.; range 4–14 years) with grass pollen hay fever were selected during springtime 1973 among out-patients at the Pediatric Department, Linköping University Hospital, Sweden. All the children had a history of hay fever during two grass pollen seasons or more, and all of them had used anti-histamines with insufficient relief and/or had developed unwanted side-effects from such therapy. Twenty-three of the children had other allergies besides the grass pollen allergy (against animal dander, dust and birch pollen). No patient had been submitted to HS therapy prior to the study.

Diaries

Special diary cards were used for the registration of symptoms and the use of drugs during the months of June to August over a period of 4 years, i.e. during one season before and three seasons after the start of specific HS. Scores were recorded for the following symptoms: nasal obstruction, sneezing, nasal discharge, itchy eyes, cough, wheezing and disturbed night's sleep, as well as any other definite allergic symptom. Each symptom was scored from 0 to 4 according to the intensity and duration of the symptoms: 0 indicating absence of symptoms and 4 indicating heavy symptoms lasting more than 4 hr. A summing-up was made for each individual symptom per season. Furthermore, a total symptom score per season was calculated for each child.

Extracts and in vivo tests

Two aqueous preparations of Timothy pollen (*Phleum pratense*) from different manufacturers were used throughout the study, i.e. both for skin and provocation tests as well as for the HS. The stock solutions were kept at 4°C and the same batches were used during the whole period. Prick tests were performed with 1/10 w/v concentrations of both extracts and reactions were compared with that of a histamine solution of 1 mg/ml (Aas & Belin, 1972). The mean size of the prick test reactions elicited by the two extracts was used in the calculations. Conjunctival provocation tests were similarly done with both extracts, but on different days. The provocation started with the instillation of one drop of diluent. If no reaction occurred, the allergen, diluted 1/100,000 w/v, was instilled. The test was continued until the concentration of 1/10 w/v was reached or until a positive reaction (increased vascularity or local itch) was elicited.

In vitro tests

Blood samples were drawn before the start of the HS (day 0) and 1, 3, 7, 14 and 30

days after its start. Serum samples were kept frozen at -20°C and analysed simultaneously. Analyses were performed in duplicate.

Total serum IgE was analysed using Phadebas IgE PRIST® and results expressed in kU/l (Cesca & Lundkvist, 1972; Johansson, Berglund & Kjellman, 1976). Timothy-specific IgE antibodies were determined with a radioallergosorbent technique using Phadebas RAST® (Johansson, Bennich & Berg, 1971). The RAST results were expressed in Phadebas RAST units (PRU), obtained from a comparison with reference sera in order to show even smaller changes in IgE antibody levels than would have been recognized if Phadebas RAST classes had been applied for the evaluation.

Timothy-specific IgG antibodies were determined using an ammonium sulphate precipitation technique and the antibody quantities were expressed as reciprocal values of the serum dilution that caused 20% precipitation of the added antigen (Ahlstedt *et al.*, 1975).

Hyposensitization treatment

A rush HS was performed with the children hospitalized for about 1 week (Berg & Johansson, 1971) during the off-season period of 1973–74. Increasing doses of allergen were given subcutaneously every 2 hr until the development of mild generalized symptoms (in four children) or local infiltrations that exceeded 3×3 cm at the site of the injection. The HS was then continued on an out-patient basis with monthly injections. An attempt was made to keep the dosage just below the threshold for general and/or marked local reactions at each injection. Hence the dose was increased whenever possible. Eight of the children were also submitted to hyposensitization with birch pollen.

Statistics

The student's *t*-test and the Wilcoxon rank sum test were used in the calculations. Because of the positive skew distribution of total IgE, IgE antibodies and IgG antibodies, their natural logarithms were used in the calculations.

Results

The total symptom score was lower in eighteen out of twenty-nine children during the third season after the HS as compared with the season before the HS (Table 1). The initial findings in these eighteen children were compared with those of the eleven children without clinical improvement (Table 2). There were no significant differences between the groups with regard to age, coexistent allergies, antibody levels before the HS, symptoms (total and individual symptom scores), the use of antihistamines or the

Table 1. Change of total symptom scores

Seasons in relation to hyposensitization	Total score		Total number of children	Children with lower scores before HS
	$\bar{x}$	s.d.		
Before	354.6	195.9	30	—
First	281.9	262.3	27	20
Second	330.0	229.7	27	14
Third	264.9	264.6	29	18

Table 2. Comparison of initial findings and clinical effect of the HS during its third season

Class	Improved	Not improved	P
No. of children			
Total	18	11	—
With more than one allergy	14	9	n.s.
Also hyposensitized against birch pollen	4	4	n.s.
Age, mean ($\pm$ s.d.)	9.3 (2.4)	9.4 (2.6)	n.s.
ln* IgE, mean ($\pm$ s.d.)	5.9 (1.1)	6.6 (0.9)	n.s.
ln IgE antibodies, mean ($\pm$ s.d.)	3.2 (2.0)	4.0 (1.8)	n.s.
ln IgG antibodies, mean ($\pm$ s.d.)	3.7 (0.8)	3.9 (0.7)	n.s.

* ln = Natural logarithm.

in vivo test results (skin and conjunctival tests). The final dose of the rush HS showed a great dispersion from 0.2 ml 10^{-2} to 0.2 ml 10^{-9} w/v allergen concentration, with no significant difference between the groups studied.

An increase of the total serum IgE (10% or more during the rush HS) occurred more often in children with a subsequent alleviation of symptoms than in those who did not improve (Table 3). The predictive value of the early IgE determinations was obvious during the second season after the HS ($P < 0.05$) and was even more pronounced in the third season; twelve out of fourteen children with an initial rise in the total serum IgE level of 10% or more then showed a decrease in total symptom scores as compared to two out of eight without such an initial rise in the IgE level ($P < 0.01$). In eighteen out of the twenty-two children (81.8%) in whom an evaluation was possible a correct prediction could be made.

The serum level of Timothy-specific IgE antibodies increased in eighteen children during the first 24 hr of the HS (Table 3). During the whole rush HS period the levels of IgE antibodies against Timothy allergens increased in twenty-one out of thirty children (70.0%). However, there was no significant difference with regard to the number of children with increasing IgE antibody levels between the groups with and without subsequent improvement. Nor was any predictive information obtained from the determinations of specific IgG antibodies or from the calculated ratio between Timothy-specific antibodies of the IgG and IgE classes.

Discussion

Repeated serum IgE determinations—before and 7 days after the start of the HS—made it possible to predict the benefit of a prolonged HS therapy. No other *in vitro* or *in vivo* tests, nor any symptoms in the case histories gave a similar predictive information.

Hence it is possible, to a certain extent, to use determinations of serum IgE levels during the rush HS for the prediction of the long-term effect of the HS therapy. If no significant elevation in the serum IgE level is achieved, the therapy should be reconsidered: the allergen extract may be poor, the dose too low or the allergy diagnosis perhaps is not well established.

Our finding confirms what others have suggested (Johansson, Berg & Foucard, 1972). In their experience, the best clinical effect of HS treatment was found in patients

Table 3. Predictive value of initial immunoglobulin levels in relation to the effect of the HS during its first three seasons

Immunoglobulin	Samples compared (day no.)	Rise*	Symptom scores per season as compared with scores before the HS					
			First season		Second season		Third season	
			Lower	Higher	Lower	Higher	Lower	Higher
Total IgE	0, 7	Yes No	11 5	4 3	9 1	6 6	12 2	2 6
<i>P</i>				n.s.†		<0.05		<0.01
IgE antibodies	0, 1	Yes No	6 4	12 1	5 5	7 7	7 4	10 5
<i>P</i>				n.s.		n.s.		n.s.
IgE antibodies	0, 7	Yes No	9 4	10 2	7 6	5 6	8 5	9 4
<i>P</i>				n.s.		n.s.		n.s.
IgE antibodies	0, 30	Yes No	5 2	10 3	5 4	4 6	6 3	7 6
<i>P</i>				n.s.		n.s.		n.s.
IgG antibodies	0, 30	Yes No	9 6	5 2	8 3	5 5	7 7	5 3
<i>P</i>				n.s.		n.s.		n.s.
Quotient IgG/IgE antibodies	0, 30	Yes No	10 6	5 2	9 2	5 6	8 5	7 3
<i>P</i>				n.s.		n.s.		n.s.

* A rise in the antibody level indicates an increase of 10% or more from the initial level.

† n.s., Not significant.

showing a significant IgE response in the initial period of HS, followed by a decrease during subsequent long-term treatment. The initial increase in the total IgE level shows that the immunological system of the patient is stimulated by an adequate allergen. It has been suggested that during an HS the amount of allergen should be increased until such an IgE response is obtained.

The booster effect of allergen injections on the production of specific reaginic (IgE) antibodies is well known (Sherman, 1941; Berg & Johansson, 1971). In our study a majority of the children showed a significant rise in specific IgE antibodies as early as 24 hr after the first injection of a minute dose of Timothy pollen extract ($0.1 \text{ ml } 10^{-8} \text{ m/v}$). The specific IgG antibodies were only measured at day 0 and 30 when most children had higher IgG antibody levels than before the HS, as could be expected from studies by Sherman (1941). However, no predictive information was obtained from the analyses of the IgG antibodies, nor from the calculated ratio between the IgG and IgE antibodies, which should have been expected from findings by Lichtenstein *et al.* (1973). Others have also failed to find a correlation between the serum levels of specific IgG antibodies and the clinical effect of immunotherapy (Brostoff, 1973; Reisman, Wypich & Arbesman, 1975; Devey, Wilson & Wheeler, 1976).

The clinical symptoms registered during the second season after the start of the HS (in 1975) were to a certain degree affected by a high pollen load during this season. The third season, being more representative regarding the pollen load, was selected for the calculation of the clinical effect. Eighteen out of twenty-nine children were improved. This is in accordance with the first results of HS, presented in 1911 (Noon, 1911). Noon & Freeman (1911) recommended a comparison of the end doses of titrated conjunctival provocation tests before and after an HS procedure as a suitable means of checking the treatment. However, even when there was a tenfold or greater increase in the conjunctival resistance of a patient after the HS, some therapeutic failures occurred. Furthermore, the method is time-consuming and annoying to the patient and is therefore not recommended.

The effect of HS therapy can be explained in several ways. In the long-term phase it is partly due to decreasing levels of allergen-specific IgE antibodies. Cellular changes in sensitivity and reactivity are also important (Levy *et al.*, 1971) as well as changes in antibody activity (Ahlstedt *et al.*, 1975). Such changes seem to occur early during an HS. The symptoms of the patient are, of course, also influenced by differences in the pollen load and the use of symptomatic as well as prophylactic drugs (Johnstone, 1975). The ideal allergen extract (Foucard & Johansson, 1976), giving a massive protection without initial stimulation of IgE-producing cells, still remains to be produced. Until this occurs, our results indicate that the efficiency of HS treatment may be evaluated during the rush HS phase by repeated determinations of total serum IgE with PRIST or other sensitive methods (Johansson *et al.*, 1976).

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