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HYPOSENSITIZATION OF PATIENTS
WITH ALLERGIC RHINITIS BY INTRANASAL
ADMINISTRATION OF CHEMICALLY MODIFIED
GRASS POLLEN ALLERGEN

A Pilot Study

By

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Immunotherapy has been used for more than 60 years in patients with allergic rhinitis (21). The mechanism underlying the clinical improvement that is often achieved by such treatment is still unclear. One factor that might contribute to this effect in parenteral hyposensitization is the occurrence of high levels of so-called blocking antibodies (3). These are considered to belong mainly to the immunoglobulin class G (16, 17).

A good allergen extract for hyposensitization should stimulate a high production of blocking antibodies without giving any local or general side effects. With a view to obtaining such allergen solutions, depot preparations with delayed absorption of the allergen (5, 8, 15) have been developed. Further efforts have been made, by modifying an allergen with different chemical substances (15, 18, 20, 26), to reduce its allergenicity, i.e. its symptom-evoking properties, while at the same time retaining its immunogenicity, i.e. its ability to initiate an immune response. The term allergoid has been applied to such preparations (18).

Treatment of timothy grass pollen extract with glutaraldehyde has been found to give an allergoid with ability to stim-

ulate the production of allergen-specific antibodies in experimental animals (20, 26). These antibodies had a blocking effect on the weal and flare reaction elicited by untreated timothy extract injected intracutaneously in patients allergic to timothy grass pollen. A mixed grass pollen glutaraldehyde-treated allergen has also been shown to stimulate the production of allergen-specific IgG antibodies in man (6, 15).

Investigation on the effect of parenteral hyposensitization with a mixed grass pollen glutaraldehyde-treated allergen has shown that clinical improvement has occurred to approximately the same extent as with the use of other conventional allergen preparations (15), but that the side effects of the injections have diminished considerably.

Local intranasal hyposensitization in allergic rhinitis has been tried previously with some success (2, 19, 23, 25). Conventional aqueous extracts were used, however, and a number of local reactions occurred; careful individual dosage was therefore required. Thus certain advantages should be gained by the use of an allergoid for this type of hyposensitization.

The present pilot study was undertaken to see how a small number of patients with allergic rhinitis due to timothy pollen would tolerate local application of a mixed grass pollen glutaraldehyde-treated allergen. The clinical effect of such treatment of rhinitis symptoms during the grass pollen season was studied and related to changes in the concentrations of allergen-specific IgG, IgA and IgE antibodies in nasal secretion and serum.

MATERIAL AND METHODS

The study comprised five adult patients (mean age 29 years, range 22-37 years) with allergic rhinitis due to timothy pollen. The diagnosis was based on a positive case history, a positive reaction to nasal provocation with timothy pollen extract, and a positive serum RAST (radioallergosorbent test) (28) against timothy pollen. In three of the patients allergic rhinitis was also evoked by one or more other allergens. None of the five patients had asthmatic symptoms. One patient had previously been given conventional parenteral hyposensitization with grass pollen extract over a period of 3 years, ending 2 years before the start of the present study.

Local nasal hyposensitization was carried out in two periods. The first trial (the winter study) took place in December-January, when there was no natural exposure to pollen, with the special purpose of testing how the allergen preparation was tolerated by the patients. The treatment was by self-administration. Using a squeeze-bottle, the patient sprayed a chemically modified grass pollen allergen directly onto the nasal mucosa. This allergoid consisted of a glutaraldehyde-treated tyrosine-adsorbed grass pollen extract (Beecham Research Laboratories, Sussex, England (15) suspended in physiological saline containing a water-soluble preservative (Phenol) and a bacteriostat (Disodium hydrogen orthophosphate). The treatment was continued for a total of 5 weeks, the dose being increased each week (Table 1). The solution was sprayed into each nostril once every other day. The estimated total dose was about 80,000 Noon Units, equivalent to about 50,000 Protein Nitrogen Units.

TABLE 1
Dosage Scheme of Local Intranasal Hyposensitization in the Winter Study.

Week of therapy	Allergoid concentration of the spray solution. Noon Units	Total daily allergoid dose. Noon Units
1	400	200
2	1,300	650
3	4,000	2,000
4	12,000	6,000
5	24,000	12,000

The second trial (the summer study) was made during the grass pollen season, in May-August, with the purpose of evaluating the clinical effect of the therapy. During this period (total 14 weeks) the treatment was the same as in the winter study, but with a different dosage scheme. In the first 4 weeks the dose was the same as in the winter study (Table 1), but during the following weeks the same dose as in the 4th week was given. The calculated total amount of allergoid given was about 200,000 Noon Units, equivalent to about 130,000 Protein Nitrogen Units. All patients had access to an oral antiallergic agent (Brompheniramine maleate and Phenylpropanol hydrochloride) to use when necessary during the pollen season.

Nasal (5) and conjunctival provocation tests were performed on four occasions (Fig. 1)—in the winter study immediately before and 1 day after the hyposensitization period and in the summer study immediately before and about 6 weeks after the end of this period. Conjunctival provocation tests were performed by instilling increasing concentrations of the allergen extract into the conjunctival sacs, and only generalized conjunctivitis was regarded as a posi-

tive reaction. An aqueous extract of timothy grass pollen (Vitrum Laboratories, Stockholm, Sweden) was used in these tests. The same batch of allergen solution was used at provocation before and after the winter studies, while another batch was used before and after the summer trials. The stock solution (10^{-1} w/v) of the respective allergen extracts was stored at $+4^{\circ}\text{C}$, and fresh dilutions were used on each test occasion.

Samples of venous blood and nasal secretion were taken on the same four occasions as the provocation tests (Fig. 1). The secretion was collected by the nasal spray washing method described previously (12). Both the nasal secretion and serum samples were stored at -20°C pending analysis. The analysis of the secretions was done on the liquid phase as described earlier (12). Albumin was determined by the single radial immunodiffusion technique (14). IgE was determined by the radioimmunosorbent test (RIST) (29), using sequential addition of the reagents as previously described (27). The results were expressed in units/ml, using the WHO preparation 68/341 as reference. IgE antibody (reagin) to timothy allergen was determined by RAST as described in detail elsewhere (11). Allergen was coupled to CNBr-activated filter paper discs, an immunosorbent-purified anti-IgE with $\text{De}2$ specificity (1) was used and the results were expressed in parts per thousand (‰) of a birch allergen reagent reference system.

IgG and IgA antibody to timothy allergen were determined by a technique based on the adsorption of the respective immunoglobulin to Sepharose, followed by the addition of ^{125}I -labelled allergen (13). For the measurement of IgG antibody, a complex of Sepharose and Staphylococcus protein A (Seph-pA) (Pharmacia Fine Chemicals, Uppsala, Sweden) diluted 1/150 (6), was used. IgA antibody was measured by the use of a 1/25 dilution of a Sepharose anti-IgA complex (Seph-aA) prepared by coupling 15 mg of Na_2SO_4 -precipitated gamma globulin fraction of specific rabbit anti-IgA (serum IgA was used as the antigen; for details, see Johansson et al. 1968) to 1 g dry CNBr-activated Sepharose 4 B (Pharmacia Fine Chemicals, Uppsala).

Twenty microlitres of a 5 mg/ml solution of Group 3 bands of timothy pollen allergen (kindly supplied by Mr B. G. Overell, Bencard, London) was labelled with 2 mCi ^{125}I as described (6). Labelled allergen corresponding to approximately 100,000 counts per min (ct/min) was added to each test tube.

For determination of IgG antibody the serum and secretion samples were diluted 1/10 in test buffer (0.1 M phosphate buffer pH 7.4, containing 0.2 per cent bovine serum albumin, 0.1 per cent "Tween 20" and 0.05 per cent NaN_3). At higher dilutions a diluent consisting of a 10 per cent solution of a normal serum in test buffer was used. When IgA antibody was determined both serum and secretion samples were tested undiluted. A few secretions had to be tested diluted 1/2–1/5, buffer being used as diluent.

The amount of radioactivity bound to the Seph-pA and Seph-aA showed a positive correlation to the concentration of IgG and IgA antibody, respectively, in the sample. The results for IgG antibody were expressed in parts per thou-

sand ($\%$) of a reference system consisting of serial dilutions of a pool of sera from patients extensively treated by parenteral hyposensitization with grass pollen extracts. IgA antibody levels were expressed in per cent of the activity of the same pool undiluted. As the cut-off value for a positive result, approximately 1.5 times the ct/min activity of a normal serum or secretion was used.

Different allergen batches were used for tests *in vitro* and *in vivo*.

The technique for collection of secretion samples involved dilution of an unknown magnitude, and to allow relevant mathematical comparisons the immunoglobulin and antibody concentrations in the secretion were expressed in relation to albumin, as described earlier (12).

RESULTS

The winter study showed that the patients were able to manage the technique of self-administration, with spraying from the squeeze-bottles, very well. The high viscosity of the strongest allergoid suspension, due to the presence of tyrosine, meant, however, that the patients had to spray up to 25 times

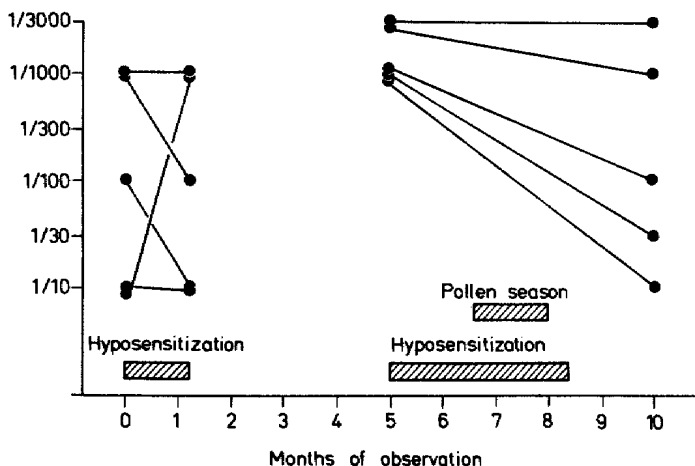


Fig. 1.

Results of nasal provocation tests before and after local nasal hyposensitization in the winter study (left half of Figure) and in the summer study (right half). The results are expressed as the highest allergen dilution (w/v) giving a positive reaction.

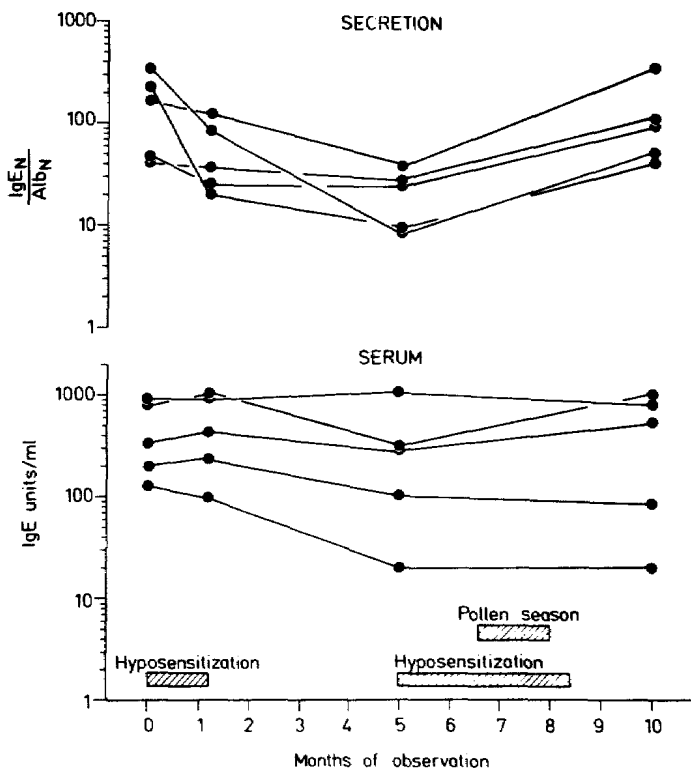


Fig. 2.

Variations of IgE levels in serum and nasal secretion during local nasal hyposensitization. In secretion, the results are expressed as the ratio nasal IgE (IgE_N)/nasal albumin (Alb_N) concentration.

into each nostril per day to obtain the recommended dose. All patients nevertheless completed the trial. There were no serious side effects, but sneezing following the spraying occurred in all patients on some occasions. Neither nasal (Fig. 1) nor conjunctival provocation tests revealed any unequivocal tendency to altered sensitivity during this winter trial period.

During the winter study the serum level of IgE increased

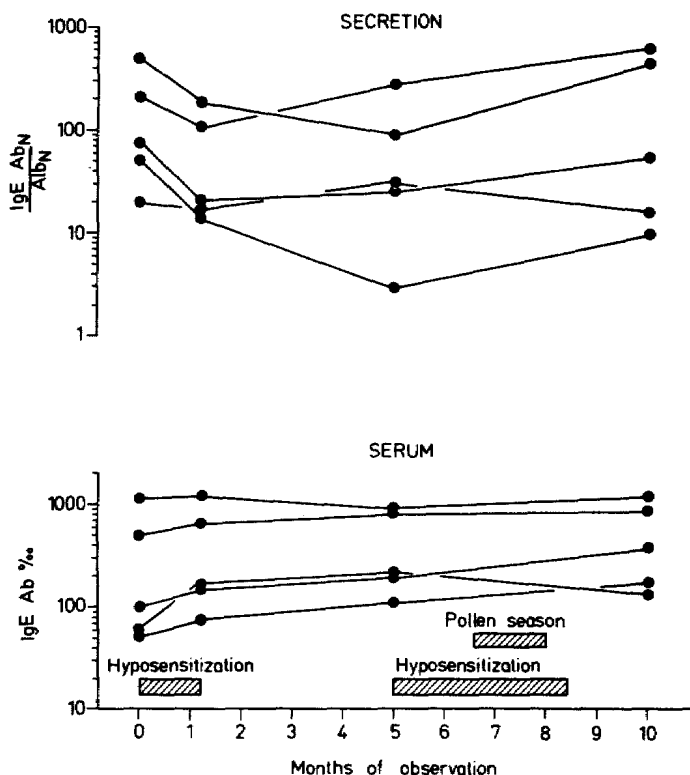


Fig. 3.

Variations of the concentrations of IgE antibody (IgE Ab) to timothy pollen in serum and nasal secretion during local nasal hyposensitization. In serum the results are expressed as % of a reference serum and in secretion as the ratio nasal IgE antibody (IgE Ab_N)/nasal albumin (Alb_N) concentration.

in three patients (Fig. 2), while the serum level of timothy-specific IgE antibodies increased in all of them (Fig. 3). In the nasal secretion both the IgE/albumin ratio (Fig. 2) and the IgE antibodies/albumin ratio (Fig. 3) decreased in all patients. Neither in serum nor in secretion did the concentrations of timothy-specific IgG and IgA antibodies show any

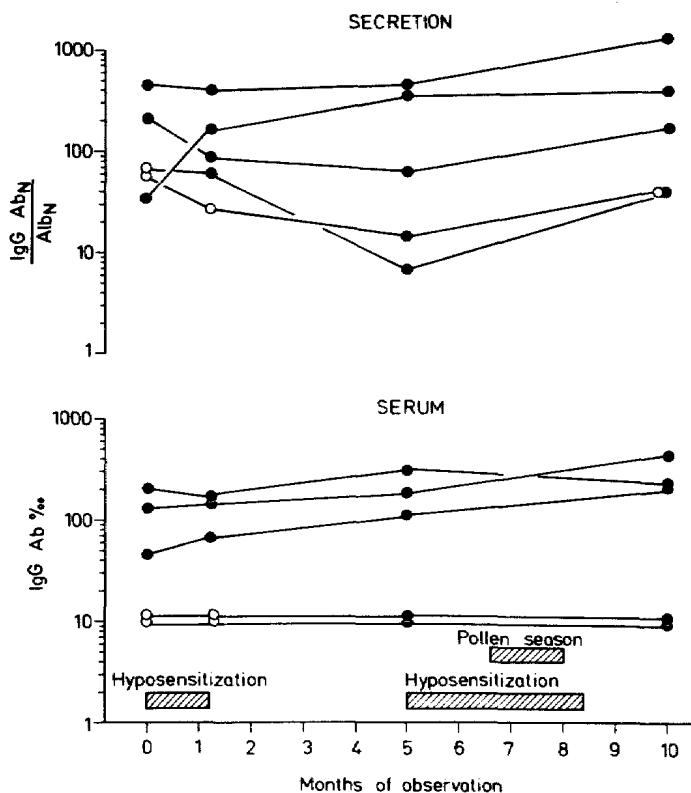


Fig. 4.

Variations of the concentration of IgG antibody (IgG Ab) to timothy pollen in serum and nasal secretion during local nasal hyposensitization. In serum the results are expressed as ‰ of a reference serum and in secretion as the ratio nasal IgG antibody (IgG Ab_N)/nasal albumin (Alb_N) concentration. When no IgG antibodies were detected the maximum level or ratio that could have been present is indicated by an unfilled circle.

definite tendency to increase during the winter study (Figs. 4 and 5).

All patients carried out the treatment during the summer study without any technical difficulties. No serious side effects

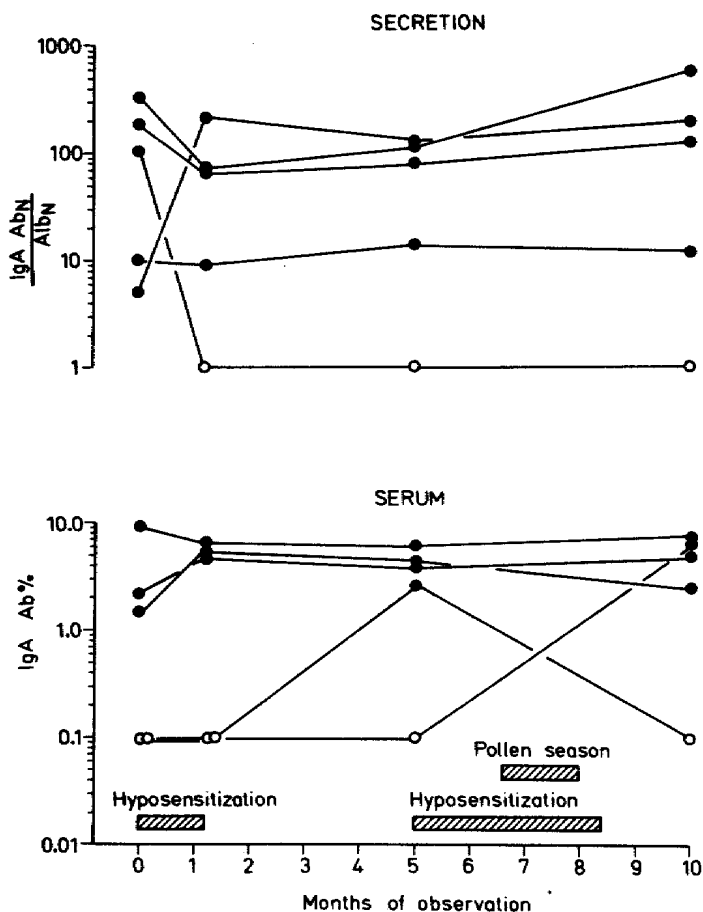


Fig. 5.

Variations of the concentration of IgA antibody (IgA Ab) to timothy pollen in serum and nasal secretion during local nasal hyposensitization. In serum the results are expressed as per cent of a reference serum and in secretion as the ratio nasal IgA antibody (IgA Ab_N)/nasal albumin (Alb_N) concentration. When no IgA antibodies were detected the maximum level or ratio that could have been present is indicated by an unfilled circle.

were noted, but two patients reported slight nasal irritation on spraying (Table 2). Four of the five patients considered that they had an easy grass pollen season and that this was due to the nasal spraying. Compared with the fifth patient, who was troubled by symptoms, these four only took a small number of anti-allergic tablets during the pollen season (Table 2). The patient who did not consider the treatment beneficial was the one in whom conventional parenteral hyposensitization had been completed 2 years prior to the study.

TABLE 2

Clinical Data in Connection with Local Intranasal Hyposensitization in the Summer Study.

Patient No.	Patient's opinion of treatment	Number of anti-allergic tablets taken during the entire pollen season	Side effects from spray	Decrease in nasal sensitivity, titre steps
1	Success	20	Some sneezing after strongest spray	3
2	Failure	80	0	0
3	Success	2	Some sneezing and rhinorrhea after weakest spray	1
4	Success	0	0	4
5	Success	2	0	2

After the summer study provocation tests showed reduced nasal sensitivity compared with the pre-seasonal results in the four patients who had had a better pollen season (Fig. 1 and Table 2). Conjunctival provocation tests before and after the same period revealed no unequivocal tendency to altered sensitivity.

The serum level of IgE showed an increase in two patients during the summer study (Fig. 2) and the serum level of timothy-specific IgE antibodies increased somewhat in four patients during the same period (Fig. 3). In the nasal secretion, on the other hand, both the IgE/albumin and the IgE antibodies/albumin ratios increased markedly in most cases during this period (Figs. 2 and 3). The concentrations of timothy-specific IgG and IgA antibodies increased moderately in both serum and secretion in three patients during the summer period (Figs. 4 and 5).

DISCUSSION

In the early part of the 1950s Herxheimer (9, 10) observed that after inhalation of repeated doses of an allergen solution in aerosol form, patients with allergic asthma showed decreased bronchial sensitivity to that particular allergen. Swineford et al. (23) confirmed these findings and also demonstrated clinical improvement during the pollen season in patients with allergic rhinitis.

Not until recent years have further reports been published on local hyposensitization of patients with allergic rhinitis (2, 19, 25). Taylor et al. (24, 25) succeeded in reducing the nasal sensitivity to grass pollen, measured by provocation titration, by the use of conventional allergen extracts. The local allergic reaction caused by this treatment could be prevented in most patients by pretreating the nose with local application of SCG (sodium chromoglycate). No serious side effects of this treatment have been reported, except in one patient, in whom it was strongly suspected that the therapy had caused systemic illness and proteinuria (7). This patient recovered completely, however, on discontinuation of the treatment.

The present pilot study was undertaken to find out how direct application of an allergoid to the nasal mucosa was tolerated by patients with allergic rhinitis, and also to attempt to evaluate the effect of such treatment on nasal sensitivity and rhinitis symptoms during the pollen season. The validity

of the decrease in nasal sensitivity obtained in the summer trial is open to criticism. Despite all efforts at objectivity the design of the trial cannot exclude an exaggeration of the trend due to an unconscious influence on the evaluation of the nasal end-point titre. However, two findings support the objectivity; firstly, the lack of a decrease of the conjunctival sensitivity in the summer trial, and secondly, the fact that the stable nasal titre occurred in the patient who was previously treated by parenteral hyposensitization.

On parenteral hyposensitization with conventional allergen extracts or allergoids, the serum level of allergen-specific antibodies of the IgG (4, 6, 17) and also of the IgA type is greatly elevated (4). These antibodies are assumed to have a blocking effect on the atopic allergic reaction (3, 17) and are therefore often called blocking antibodies. After parenteral hyposensitization, allergen-specific IgG and IgA antibodies have also been found in nasal secretion from patients with allergic rhinitis (4, 22), but the concentrations have been negligible compared with those in serum (4).

In order to achieve a maximal clinical effect in allergic rhinitis, hypothetically, it should be more essential to have a high concentration of blocking antibodies in the nasal secretion than in the serum. Admittedly, the level of timothy-specific IgG and IgA antibodies increased in both the serum and nasal secretion in some of our patients, but this increase was not nearly as great as has been found previously in serum on parenteral hyposensitization (4).

This study of local hyposensitization with an allergoid in five patients with allergic rhinitis is much too limited to allow any definite conclusions to be drawn. However, the clinical improvement that was observed in four patients and the fact that the treatment was well tolerated without any marked side effects at the same time as the allergoid showed immunogenic properties, seems promising. Further trials of local intranasal hyposensitization with allergoids in a larger series of patients, including an adequate control group, are accordingly in progress.

SUMMARY

In a pilot study, five adult patients with allergic rhinitis due to grass pollen underwent local intranasal hyposensitization with a chemically modified grass pollen extract—a so-called allergoid. Local hyposensitization during a pre-seasonal period resulted in an increased serum level of timothy-specific IgE antibodies in all patients, indicating that the allergoid had immunogenic properties. In four of the patients the clinical effect during the grass pollen season was judged satisfactory. Pre- and post-seasonal provocation test in these four patients also showed a reduction of the nasal sensitivity during this period. All patients tolerated the treatment well without any marked side effects. These promising preliminary results motivate further investigation into this form of therapy.

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