

IgE, IgG and IgA antibodies in serum and nasal secretion during parenteral hyposensitization

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Summary

Eighteen adult patients with allergic rhinitis due to Timothy pollen were observed for 36 weeks before, during and after the grass pollen season. Eight patients were treated by parenteral hyposensitization with grass pollen extract, and ten patients who were given no immunotherapy served as controls. Timothy-specific IgE, IgG and IgA antibodies in samples of serum and nasal secretion were quantified by radioimmuno-logical technique.

In comparison with the control group, the serum concentration of Timothy-specific IgE antibodies increased significantly ($P < 0.05$) during the preseasonal hyposensitization treatment and then decreased significantly ($P < 0.05$) during and after the pollen season while this therapy was being continued. In the hyposensitized patients the serum concentration of both IgG and IgA antibodies increased highly significantly ($P < 0.01$ and $P < 0.001$, respectively) during immunotherapy.

In nasal secretion quantitative changes of the three types of antibodies were usually less pronounced or not detectable at all. The concentration of IgG antibodies, however, showed some increase in the nasal secretion during hyposensitization. These minor increases in allergen-specific IgG and IgA antibodies in nasal secretion might explain why parenteral hyposensitization in allergic rhinitis often does not give complete relief from symptoms.

Introduction

Parenteral hyposensitization, also called desensitization or specific immunotherapy, has been a common form of treatment of allergic asthma and rhinitis for more than 60 years (Noon, 1911). Sorenson (1976), in a critical review of several well-controlled studies, has found that, especially in pollen allergy, such therapy gives at least partial relief from the allergic symptoms.

The mechanism underlying the clinical improvement in parenteral hyposensitization is not clear. One factor which is considered of importance is the occurrence of so-called blocking antibodies (Cooke *et al.*, 1935; Fagerberg, Nilzén & Wiholm, 1972), which seem to be mainly of the IgG type (Lichtenstein, Holtzman & Burnett, 1968;

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Lichtenstein, Norman & Ishizaka, 1973). It is thought that these antibodies interfere in some way with the allergen-reagin reaction, thus preventing the release of vasoactive substances from the mast cells.

Studies have shown that the serum level of blocking antibodies is considerably raised during hyposensitization (Lichtenstein *et al.*, 1973; Foucard & Johansson, 1976). No direct causal relationship between the serum level of IgG antibodies and the clinical improvement has, however, been found (Lichtenstein *et al.*, 1973).

One possible explanation for this poor correlation in allergic rhinitis is that the serum concentration of blocking antibodies is not relevant, as both the immunological reactions and the symptoms are localized in the nose. In an attempt to elucidate this question a group of patients with pollen-induced allergic rhinitis during a course of parenteral hyposensitization has been studied. The aim of the investigation was to follow the occurrence and change of allergen antibodies of different immunoglobulin classes in the serum and nasal secretion. The clinical effect of the therapy was not evaluated.

Materials and methods

The study comprised twenty-one adult patients with allergic rhinitis due to Timothy pollen. There were three patients who failed to complete the study, leaving a group of eighteen patients (mean age 26 years, range 17–37 years). In addition, five healthy subjects without any history of allergy served as a reference group for determination of normal antibody levels.

The allergy diagnosis was based on a positive case history, a positive intracutaneous test and a positive nasal provocation with Timothy allergen extract, and demonstration of Timothy-specific IgE antibodies in the serum by RAST (radioallergosorbent test) (Wide, Bennich & Johansson, 1967). Several of the patients also had allergic rhinitis caused by one or more other allergens. Some patients had mild asthmatic symptoms. None of the patients had previously undergone parenteral hyposensitization.

The patients were divided randomly into two groups. One group of eight patients was given parenteral hyposensitization, while the other ten patients, who did not receive immunotherapy, served as a control group. The hyposensitization was started about 18 weeks before the Timothy season. The grass pollen extract used was Allpyral five-grass mix (Dome) following the dosage scheme described in detail previously (Fagerberg *et al.*, 1972). In accordance with this scheme, ten injections were given at weekly intervals in increasing concentrations, after which the maintenance dose was given at intervals of 6 weeks as perennial therapy.

All patients had access to an oral anti-allergic agent (brompheniramine maleate and phenylpropanol hydrochloride) for use when required during the pollen season.

Samples were taken on three occasions (Fig. 1a). The first ones (hereafter called week 0) were taken about 18 weeks before the Timothy season, immediately before the hyposensitization. The second ones (week 15) were taken about 3 weeks before the pollen season, after 15 weeks of hyposensitization. The third samples (week 36) were taken about 15 weeks after the end of the pollen season, after 36 weeks of hyposensitization. On each occasion one sample each of venous blood and nasal secretion were taken, the latter by the nasal spray washing method described earlier (Johansson & Deuschl, 1976). The serum from the venous blood samples and the secretions were stored at -20°C pending analysis.

Both the serum and secretion samples were thawed and carefully mixed before

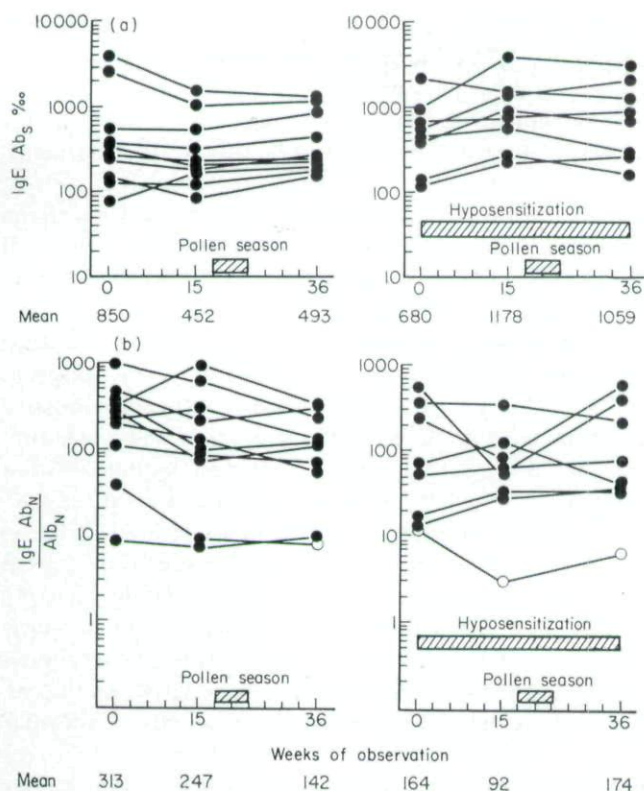


Fig. 1. (a) Serum concentrations of IgE antibodies (IgE Ab_s) in patients treated with hyposensitization and in untreated controls (left figure), expressed in % of that in a reference serum. Each point represents one patient. The arithmetic mean for all patients in each group at each observation time is given. (b) IgE antibodies in nasal secretion expressed as the ratio nasal IgE antibody (IgE Ab_N)/nasal albumin (Alb_N) concentration. When no IgE antibodies were detected in the secretion the maximum ratio that could have been present is indicated by an unfilled circle.

being tested. The analyses of the secretions were performed on the liquid phase as described previously (Johansson & Deuschl, 1976). Albumin was determined by the single radial immunodiffusion technique (Johansson, Högman & Killander, 1968). IgE was determined by the radioimmunosorbent test (RIST) (Wide & Porath, 1966), using a sequential addition of reagents as previously described (Wide, 1971). The IgE antibody (reagin) to Timothy allergen was determined by RAST, as described in detail earlier (Johansson, Bennich & Berg, 1971). The allergen was coupled to CNBr-activated filter paper discs, an immunosorbent purified anti-IgE with Dε2 specificity (Bennich & Johansson, 1970) was used and the results were expressed in parts per thousand (‰) of a birch allergen reagin reference system.

IgG and IgA antibodies to Timothy allergen were determined by a technique based on the adsorption of the respective immunoglobulin to Sepharose, followed by the addition of ¹²⁵I-labelled allergen (Johansson *et al.*, 1974). For measurement of IgG antibody a complex of Sepharose and *Staphylococcus* protein A (Seph-pA) (Pharmacia

Fine Chemicals, Uppsala) diluted 1/150 (Foucard & Johansson, 1976) 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 Na₂SO₄-precipitated γ -globulin fraction of a specific rabbit anti-IgA antiserum (human serum IgA was used as the antigen; for details, see Johansson *et al.*, 1968) to 1 g dry CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala).

20 μ l of a 5 mg/ml solution of a pool of group 3 bands of Timothy pollen allergen (kindly supplied by Mr B. G. Overell, Bencard, London) was labelled with 2 mCi ¹²⁵I as described (Foucard & Johansson, 1976). Labelled allergen corresponding to approximately 100 000 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 mol/l phosphate buffer, pH 7.4, containing 0.2% bovine serum albumin, 0.1% Tween 20 and 0.05% NaN₃). At higher dilutions a diluent consisting of a 10% solution of a normal human 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, in which case buffer was used as diluent.

The amount of radioactivity bound to the Seph-pA and Seph-aA showed a positive correlation with the concentrations of IgG and IgA antibody, respectively, in the sample. The results for IgG antibody were expressed in parts per thousand (‰) 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 as a percentage 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.

For mathematical calculations of the obtained secretion values the ratio to albumin concentration was used, according to principles described previously (Johansson & Deuschl, 1976).

Statistical methods

The change between different time points, e.g. for samples taken before and during hyposensitization, was analysed by *t*-testing. When comparing different treatment groups, consideration had to be paid to the different-sized variances in these groups, and the approximate *t*-test was used as the test formula. The use of these two tests has been described earlier (Deuschl, 1976). All tests were double-sided. For further details of the test formula used, and of the determination of the number of degrees of freedom, see Armitage (1971).

Results

In none of the sera or nasal secretions of the five healthy subjects were Timothy-specific IgE, IgG or IgA antibodies found.

In seven of the eight hyposensitized patients the serum level of Timothy-specific antibodies increased between weeks 0 and 15 (Fig. 1a), whereas in the untreated control group this level generally decreased between the corresponding time points. This difference between the two groups was significant ($P < 0.05$). Between weeks 15 and 36 the reverse was observed, with decreasing IgE antibody levels in the majority of hyposensitized patients and, in general, increasing levels in the controls (Fig. 1a). Here also the difference between the groups was significant ($P < 0.05$).

The IgE concentration in the serum showed similar changes to those of IgE

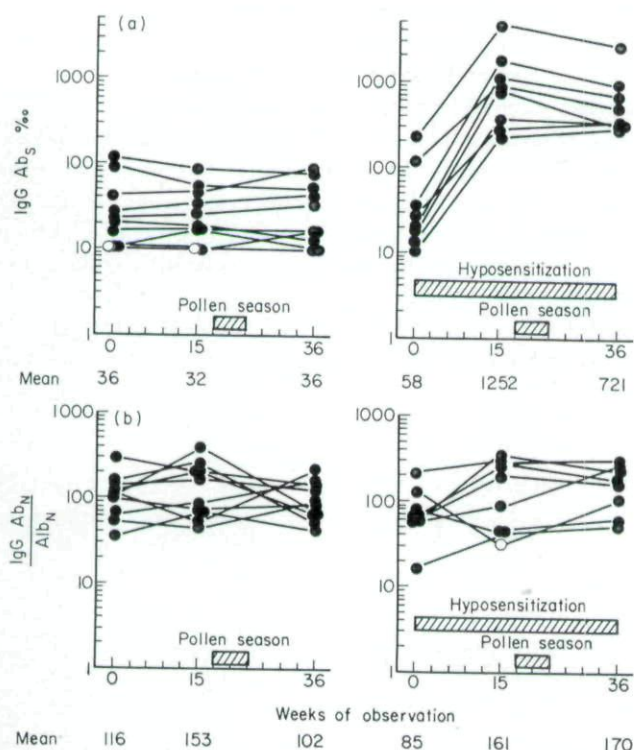


Fig. 2. (a) Serum concentrations of IgG antibodies (IgG Ab_s), expressed in % of that in a reference serum. When no IgG antibodies were detected the maximum level that could have been present is indicated by an unfilled circle. (b) IgG antibodies in nasal secretion expressed as the ratio nasal IgG antibody (IgG Ab_N)/nasal albumin (Alb_N) concentration. For notations see legend to Fig. 1.

antibodies, but less pronounced. The decrease in the serum IgE level in the hyposensitized patients between weeks 15 and 36 was significant ($P < 0.05$) in comparison with the increase in the control group.

IgE antibodies in nasal secretion, expressed as the ratio IgE Ab_N/Alb_N (IgE antibodies in nasal secretion/albumin in nasal secretion) decreased in most patients of the control group between week 0 and week 15 (Fig. 1b); this decrease was significant ($P < 0.05$). Otherwise, the ratios IgE Ab_N/Alb_N and IgE_N/Alb_N did not show any distinct changes between the different observation times in either of the groups.

All hyposensitized patients exhibited a distinct increase in the serum level of IgG antibodies between weeks 0 and 15 ($P < 0.01$) (Fig. 2a). This level then decreased to some extent in most of the treated patients between weeks 15 and 36, but at the latter time point the values were still significantly higher than at week 0 ($P < 0.01$). In the control group no tendency to a change in the concentrations of IgG antibodies in the sera was noted between the different times of observation.

The ratio IgG Ab_N/Alb_N (IgG antibodies in nasal secretion/albumin in nasal secretion) increased somewhat between weeks 0 and 15 in the majority of hyposensitized patients (Fig. 2b). Between weeks 0 and 36 this ratio increased significantly in the treated patients ($P < 0.05$). In the control group no clear tendency to a change in this ratio was noted between the different observation times.

In the serum, Timothy-specific IgA antibodies were found in only three patients at week 0 (Fig. 3a). In the hyposensitized patients, with one exception, this antibody titre increased markedly between weeks 0 and 15; a moderate decrease was then noted in six of the eight patients between weeks 15 and 36. The increase in the serum concentration of IgA antibodies between weeks 0 and 36 was highly significant ($P < 0.001$). In the control group an increase in this serum concentration was observed in three patients between weeks 15 and 36. One of the control patients deviated completely from the rest of the group, showing an initially high antibody concentration which decreased considerably after week 15. This patient gave birth to a healthy child at the estimated time of delivery between weeks 15 and 36.

In neither of the two groups of patients was there any clear change in the ratio of IgA antibodies in nasal secretion/albumin in nasal secretion between the different times of observation (Fig. 3b).

Discussion

Parenteral hyposensitization has been used since 1911 (Noon *et al.*, 1911) as a method of allergen-specific treatment of patients with atopic, allergic diseases. On examination

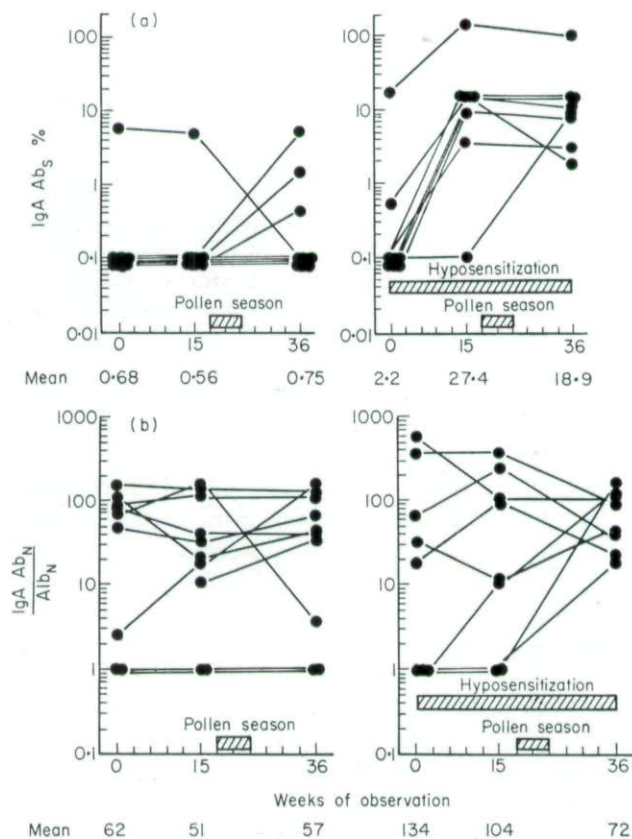


Fig. 3. (a) Serum concentrations of IgA antibodies (IgA Ab_S), expressed as percentage of that in a reference serum. All points placed at a level with or below 0.1% represent a value less than 0.1%. (b) IgA antibodies in nasal secretion expressed as the ratio nasal IgA antibody (IgA Ab_N)/nasal albumin (Alb_N) concentration. All points placed at a level with 1.0 represent a ratio less than 1.

For notations see legend to Fig. 1.

of the literature it is found that this treatment, when given to patients with pollen-induced allergic rhinitis, often leads to an alleviation of the symptoms (Sorensen, 1976), although complete freedom from symptoms is not always ensured (Fagerberg *et al.*, 1972; Lichtenstein *et al.*, 1973). The exact mechanism by which parenteral hyposensitization exerts its effect is still obscure. It has been found, however, that so-called blocking antibodies usually show an increase in the serum during hyposensitization, at the same time as the clinical allergic symptoms are ameliorated (Lichtenstein *et al.*, 1973).

Several hypotheses have been proposed to explain the effect of hyposensitization. According to one theory, blocking antibodies might be able to bind (block) the allergen so that, firstly, a smaller amount of allergen becomes directly available to the mast cell-fixed reagins, and secondly, a smaller amount comes in contact with the reagin-producing cell system. In the former case a diminished allergen-reagin reaction should result in a reduced release of mediator substances and, consequently, alleviation of the allergic symptoms. In the latter case there should be an inhibitory effect on the reagin synthesis in the plasma cells, either directly or via the T cell system. Non-immunological mechanisms are also conceivable. In the early stage of hyposensitization, parenterally administered allergen will probably give rise to a release of mediator substances. This might sometimes lead to local and even general reactions. As the mast cell probably needs several weeks to renew its capacity to release histamine (Boréus, 1961), such mild clinical reactions may lead to 'desensitization' of the mast cell, which will then become refractory to the natural allergen exposure. Another conceivable explanation for the long term effect of the hyposensitization is that the sensitivity of the shock organ to the biologically active substances released may be reduced.

The term blocking antibodies was introduced in the 1930s (Cooke *et al.*, 1935), and referred to a serum factor that is able to block the reagin-mediated allergic reaction. These blocking antibodies in serum that appear during parenteral hyposensitization are mostly of the IgG class (Lichtenstein *et al.*, 1968; Sobotka *et al.*, 1976; Wide, 1976), and some authors consider that they belong mainly to the subgroup IgG4 (Devey, Wilson & Wheeler, 1976; Giessen *et al.*, 1976).

Supporting the view that blocking antibodies are of clinical significance are the results obtained on parenteral hyposensitization against hymenoptera stings (Sobotka *et al.*, 1975; Valentine *et al.*, 1976). They gave parenteral hyposensitization with venom from the respective insects to patients who had previously sustained life-threatening anaphylactic reactions to insect stings. A good clinical effect was obtained, in that only local reactions occurred after experimental stings by the same type of insect. At the same time the authors noted an increase in all patients in the serum concentration of blocking antibodies of the IgG type to venom antigens. In this type of allergic reaction, where the allergen gains entry parenterally, the concentration of blocking antibodies in the serum thus seems to be directly correlated to the clinical protective effect, which is not always the case in allergic rhinitis (Lichtenstein *et al.*, 1973).

The present results have clearly shown that the serum concentrations of Timothy-specific IgE, IgG and IgA antibodies in patients with allergic rhinitis increase initially to a significant degree during parenteral hyposensitization. This is in agreement with the results of other investigations, where an increase in IgE (Berg & Johansson, 1971; Yunginger & Gleich, 1973; Foucard & Johansson, 1976) and IgG antibodies (Lichtenstein *et al.*, 1973; Yunginger & Gleich, 1973; Foucard & Johansson, 1976) in the

serum has been found. When conventional allergen preparations are used for hypsensitization a stimulation of the IgE antibody production clearly cannot be avoided. The fact that no clinical deterioration nevertheless occurs might be explained either by a more rapid increase in the serum content of IgG than IgE antibodies (Foucard & Johansson, 1976) or by a 'desensitization' of the mast cells due to essentially asymptomatic mediator release resulting from the hypsensitization.

Allergen-specific antibodies of both the IgG and IgA type have been found in nasal secretions from allergic patients in other investigations (Dolovich, Tomasi & Arbesman, 1970; Turk, Lichtenstein & Norman, 1970; Tse, Wicher & Arbesman, 1973; Platts-Mills *et al.*, 1976). Only Platts-Mills *et al.* have studied the effect of parenteral hypsensitization. They found that IgG and IgA allergen antibodies in nasal secretions increased in hypsensitized patients in comparison with untreated controls. However, many of the treated patients had a higher level of allergen antibodies after the pollen season than before, which indicates that in spite of the treatment the natural allergen exposure could stimulate the production of allergen antibodies.

Our results showed that during parenteral hypsensitization allergen antibodies in nasal secretion did not increase to the same extent as those in serum. Admittedly, the concentration of IgG antibodies was somewhat increased, but the level of IgA antibodies in nasal secretion remained grossly unchanged during the course of treatment. It is likely that the IgG antibodies represent a transudation product from the serum. The stable levels of IgA antibodies probably reflect an inability of the parenterally administered allergen to reach the immune system responsible for the local production of IgA (Tomasi & Bienenstock, 1968).

In patients with allergic rhinitis it would seem that a high level of blocking antibodies in the secretion or mucosa of the shock organ should be aimed at to achieve an optimal clinical effect. Direct application of the allergen to the nasal mucosa should be the most effective way of achieving local production and accumulation of blocking antibodies. Such a procedure is complicated, however, by the fact that the allergen will then induce rhinitis symptoms (Taylor & Shivalkar, 1972). Local nasal application of a chemically modified allergen, an 'allergoid', in which the immunogenic antibody-stimulating effect has been retained but the allergenic effect has been eliminated or reduced (Marsh, Lichtenstein & Campbell, 1970; Foucard & Johansson, 1976) would seem to be a practicable way of producing such local immunity.

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