

Allergen-antibody complexes can efficiently prevent seasonal rhinitis and asthma in grass pollen hypersensitive patients

Allergen-antibody complex immunotherapy

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We have prepared antigen-antibody complexes from grass pollen allergens and autologous specific antibodies isolated by immunoadsorption from the serum of allergic patients. These complexes were inoculated into patients in a double-blind trial to evaluate their effect on grass pollen-related rhinitis and bronchial asthma. Thirty-eight grass pollen-hypersensitive patients were allocated to three groups; patients in the first two groups were treated with antigen-antibody complexes at different ratios and dosages and were compared with the third group who received the placebo carrier buffer alone. In addition, we treated a fourth group who had already received antigen-antibody complex inoculation during the previous pollen season. Injections were given every 2 weeks during the pollen season, starting 5 weeks prior to it. Tolerance was excellent with no signs of local or systemic side effects. The treatment prevented nasal symptoms while enabling the patients to reduce antihistamine intake. Bronchial asthma was virtually absent in the treated groups even though no bronchodilators or corticosteroids had to be taken. Specific IgE antibodies did not increase during the pollen season nor did IgG "blocking" antibodies. Inoculation of allergen-antibody complexes could provide a valuable alternative for the treatment of immediate hypersensitivity to airborne allergens as it appears to be safe and rapidly efficacious. This treatment offers several advantages compared to conventional hyposensitization and is characterized by the absence of an increase in specific IgG antibodies.

Key words: allergen-antibody complexes; allergic rhinitis; bronchial asthma; grass pollen; immunotherapy.

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Immunotherapy of allergic diseases usually consists of regular injections of progressively increasing allergen doses (5). Such therapy is associated with an increase in the specific IgG immune response, the so-called "blocking" antibodies, which can compete with IgE antibodies for binding to allergen (15), and recent developments in this area have resulted in the production of new allergen derivatives with increased immunogenicity and reduced side ef-

fects (12). However, the relationship between clinical benefit and specific IgG levels in hypersensitivity to airborne allergens remains poorly defined (17).

Another approach to the treatment of allergic diseases is suppression of the production of IgE antibodies. This could be achieved either by isotypic or antigen-specific IgE immunosuppression. Much work has recently been carried out on the isotypic regulation of IgE in animals

(e.g. 23) but the feasibility of suppressing IgE production in man has yet to be determined. Antigen-specific suppression of IgE production has already been achieved in animal models. For instance, a primary IgE immune response can be efficiently prevented by actively produced or passively transferred anti-idiotypic antibodies (2). However, attempts to suppress an ongoing IgE immune response in animals have up to now been disappointing (8), with a few exceptions (25).

It has been known for some time that antigen-antibody complexes made in antibody excess can suppress an immune response (7) and we have investigated the application of this property of immune complexes to the treatment of immediate hypersensitivity. It was realized early on that such complexes should be formed from autologous antibodies (24), i.e., patients should be inoculated with complexes made from their own specific anti-allergen antibodies. Our previous studies (19, 21) indicated that idiotypic specificities are shared between IgE and IgG antibodies in a given individual; this opens the possibility of using autologous specific IgG antibodies to form immune complexes rather than IgE antibodies whose serum concentration is too low for easy isolation.

We have presented (16) a first double-blind clinical trial of this approach in asthmatic patients hypersensitive to house dust mite, *Dermatophagoides pteronyssinus* (Dpt). As this study and other preliminary trials (Machiels et al, 1990a, manuscript submitted) have shown that the inoculation of allergen-antibody complexes is safe and clinically effective, we have now extended this work to a double-blind clinical trial on grass pollen-hypersensitive patients.

Our results show that: (i) inoculation of complexes made from allergen and autologous specific antibodies results in a significant clinical improvement of both nasal symptoms and bronchial asthma; (ii) the treatment does not produce local or systemic side effects; (iii) clinical benefit is achieved within a few weeks of treatment; (iv) the treatment prevents the seasonal increase in specific IgE antibodies without increasing specific IgG antibodies.

MATERIAL AND METHODS

Patients

Fifty-one patients (28 males and 23 females with an average age of 30, range 12-51) took part in this study. Informed consent was obtained and the study received approval of the University Ethics Committee. Grass pollen-hypersensitivity was identified by a clinical history of seasonal allergic rhinitis for at least 3 consecutive years. Seasonal bronchial asthma was present in 60% of cases, which corresponds to the usual proportion of allergic asthma in our practice; acute conjunctivitis and urticaria affected only a minority of patients. Each patient had a total serum IgE > 20 kU/l and positive ($\geq 2+$) Phadebas RAST (Pharmacia, Uppsala, Sweden) and skin prick tests for at least three of the following grass pollens: cocksfoot, fescue, meadow, rye, timothy and sweet vernal. Allergens for skin testing were purchased from Ben-card Ltd (Epsom, Surrey, UK; batch nos 4103, 4106, 4108-4111, respectively); a test was considered positive when eliciting a wheal of a size at least comparable to the wheal obtained with 1 mg/ml histamine.

Usual treatment during grass pollen seasons consisted of antihistamine tablets, nasal sprays (cromoglycate, vasoconstrictor or flunisolide), inhaled β_2 -agonists and, in 25% of the cases, systemic corticosteroids. Twenty-four patients (47%) had been hyposensitized in the past by conventional treatment including regular inoculations of either aqueous solutions of grass pollens or alum-adsorbed allergens in increasing dosages for at least 2 consecutive years; this treatment, which was terminated at least 1 year prior to this trial, had resulted in only mild or no improvement of clinical symptoms.

Allergens

A glycerinated grass pollen extract (B2, Ben-card) was extensively dialysed against phosphate-buffered saline, pH 7.4 (PBS) containing

100 U aprotinin/ml (Boehringer, Mannheim, Germany) as protease inhibitor and concentrated by ultrafiltration on a YM10 membrane (Amicon, Danvers, MA). A stock solution containing 2 mg total protein/ml (0.25 Noon unit/ng protein) was distributed into aliquots and kept at -80°C until just before use. This B2-pollen extract, which is composed of 12 common grass pollens, was used throughout the study for both the isolation of specific antibodies by immunoabsorption and the formation of complexes for treating the patients.

Immunosorbent

B2-pollen extract was covalently coupled by carbodiimide reaction with CH-Sepharose (Pharmacia) as recommended by the manufacturer, using 2 mg of protein for each ml of Sepharose. The suspension was incubated overnight at 4°C with end-over-end mixing. Glycine (1M final concentration) was then added for 2 h at room temperature to neutralize excess reactive groups before washing successively with phosphate-buffered saline, pH 7.4 (PBS), 0.1 M sodium bicarbonate buffer, pH 8.3, and 0.1 M sodium citrate buffer, pH 4.0, the last two buffers also containing 0.5 M NaCl. A final wash was made with 3M-NaSCN and the immunosorbent was kept at 4°C in PBS containing aprotinin.

Preparation of specific anti-grass pollen antibodies

Immunoglobulins were fractionated from HIV- and hepatitis B-screened individual plasmas by Na_2SO_4 precipitation followed by extensive dialysis in PBS and gel filtration chromatography on a 6×90 cm Fractogel TSK HW-55F (Merck, Darmstadt, Germany) (20). Fractions containing IgG, IgA and IgE antibodies were pooled, concentrated to 10 mg/ml by ultrafiltration on a YM10 membrane (Amicon) and passed at a rate of 10 ml/h over a 5-ml column of B2-Sepharose. The column was then washed with 50 ml PBS followed successively by the same volume of 1M NaCl and of distilled water. Specific anti-B2 antibodies were eluted from the

immunosorbent with 20 ml of 3M-NaSCN at a rate of 60 ml/h and immediately dialysed against 100 volumes of PBS. An average of 600 μg specific antibodies were recovered from 100 ml of plasma; the isotypic distribution was 85 % IgG, 15 % IgA and 0.1 % IgE. This procedure has been described in detail elsewhere (20). For *in vitro* assays, preparations of specific antibodies from individual patients were labeled with biotin. Ten μl of N-hydroxysuccinimido-LC-biotin (Pierce, Rockford, IL) freshly diluted to 10 mg/ml in dimethylformamide was added to 0.5 ml of antibodies diluted to 200 $\mu\text{g}/\text{ml}$ in PBS. After vigorous vortexing, the mixture was incubated for 2 h at 21°C and dialysed against 0.15 M NaCl. The solution was then diluted in glycerol v/v and stored at -20°C until immediately before use.

Preparation of allergen-antibody complexes

The ratio of allergen to antibody was determined for each patient with an inhibition ELISA. Polystyrene microtitration plates were coated with 40 $\mu\text{g}/\text{ml}$ grass pollen extract in glycine buffer (pH 9.2) and incubated overnight at 4°C . The plates were washed three times with PBS containing 0.1 % Tween-20 (Technicon, Tarrytown, NY) before addition of a 100- μl aliquot of biotin-labeled specific antibodies diluted from 20 to 0.1 $\mu\text{g}/\text{ml}$ in PBS containing 0.5 % bovine serum albumin (PBS-BSA). After an incubation of 4 h at 21°C , the plates were washed as above and supplemented for 30 min at 21°C with avidin-peroxidase (Sigma, St Louis, MO) diluted to 1 $\mu\text{g}/\text{ml}$ in PBS-BSA. The final wash was followed by addition of 100 μl of a solution containing 0.02 % 2,2'-azino-bis (3-ethylbenzthiazoline)sulfonic acid (ABTS, Sigma) in phosphate-citrate buffer, pH 5.0 and 0.03 % H_2O_2 . After 15 min in the dark at 21°C , the plates were read at 405 nm with a Kontron 210 spectrophotometer (SLT, Vienna, Austria).

For inhibition assays, we used the lower concentration of biotin-labeled specific antibodies which resulted in a maximal binding to the plate; it corresponded to 1 $\mu\text{g}/\text{ml}$ in each case.

Table 1.
Antigen-antibody complex immunization schedule

Day	Volume (μ l)	Groups 1 & 2		Group 3	
		Antibody (μ g)	Antigen (μ g)	Antibody (μ g)	Antigen (μ g)
0	20	4	80	0.08	8
15	40	8	160	0.16	16
30	80	16	320	0.32	32
45	160	32	640	0.64	64
60	300	60	1200	1.2	120
75	400	80	1600	1.6	160
90	400	80	1600	1.6	160
Total		280	5600	5.6	560

The grass pollen extract was diluted serially in PBS-BSA and a 150- μ l aliquot of each dilution was mixed with an equal volume of 2 μ g/ml biotin-labeled specific antibodies for 30 min at 37°C. 100 μ l of this mixture was then added to grass pollen-coated plates for 4 h at 21°C, followed by addition of avidin-peroxidase and ABTS as described above. The concentration of grass pollen which inhibited 20% of the specific antibody binding to the plates was selected to calculate the allergen-antibody ratio. It corresponded to an average of 1/10 (w/w), the ratio selected for treating Group 3 patients (see below, patient randomization). To determine whether a further increase in specific antibodies could improve the therapy, patients of Groups 1 and 2 were inoculated with a 1/50 allergen-antibody ratio.

Allergen and individual specific anti-allergen antibodies were diluted in sterile saline (9 g/l) containing 0.3% human serum albumin and 0.4% phenol and mixed at a predetermined ratio (see below, patient randomization and treatment) for 30 min at 21°C. Allergen-antibody complexes were then distributed into aliquots and kept at -80°C until just before use.

Patient randomization and treatment

Patients were allocated to one of four groups. The 13 patients of Group 2 had already been treated the previous year with complexes containing 400 ng allergen and 4 μ g allergen-specific antibody per ml (Machiels et al, 1990a, manuscript submitted). The other 38 patients

were randomly allocated to the three remaining groups with randomization adequacy being confirmed by checking that no differences existed between groups as for previous clinical history, incidence of asthma, previous treatment, allergen sensitivity or number of individuals who had been hyposensitized in the past. Groups 1 and 2 (13 patients in each case) were inoculated with immune complexes at a 1/50 ratio of allergen to specific autologous antibodies (4 μ g allergen and 200 μ g antibody/ml). Group 3 (13 patients) received complexes at a 1/10 ratio (400 ng allergen and 4 μ g antibody/ml). Group 4 (12 patients) were inoculated with carrier buffer alone, i.e., 0.3% human serum albumin and 0.4% phenol in 9 g/l saline.

Immunization schedule

Treatment was administered between April 15 and July 15, which in Belgium corresponds to 5 weeks before grass pollen season until its end. Injections were made intradermally at 2-week intervals, following the immunization schedule of Table 1. Local wheal areas were measured after 15 min. The patients were asked to report any local reaction occurring 6 to 12 h after inoculation. Each injection was performed by the same physician (J.M.) in a double-blind protocol.

Clinical evaluation

Patients were asked to fill in a diary card which listed the evaluation of nasal (obstruction,

rhinorrhea and sneezing), ocular (pruritus and watering), respiratory (shortness of breath, cough and wheezing) and cutaneous (itching and urticaria) symptoms. Each symptom was graded on a discontinuous scale from 0 (no symptom) to 4 (very severe symptoms). Drug intake was monitored on a day-to-day basis. Antihistamine tablets (terfenadine) and a pressurized aerosol of bronchodilator (salbutamol) were provided to the patients at the beginning of the study, together with instructions for use. When symptoms were not adequately controlled by one of these drugs alone or in combination, the patients immediately contacted the physician in charge (J.M.) who decided on a course of corticosteroids. In addition a 100-mm visual analogue scale (VAS) was used to follow patients' general state of well-being (6).

Analysis of specific anti-grass pollen antibodies

Five-ml aliquots of serum collected at each injection date during the study were salt precipitated and fractionated by gel filtration (20). IgG containing fractions were passed over a B2-coupled Sepharose column. Total IgG and IgE antibodies were quantitated in the eluted fractions by isotype-specific ELISA (Machiels et al, 1990b, manuscript submitted).

Pollen counts

Concentrations of grass pollen grains/m³ were evaluated daily using a Burkard Volumetric Trap (courtesy of Dr. N. Noland, Institut d'Hygiène et d'Epidémiologie, Brussels, Belgium).

Statistics

In each group, symptom scores were averaged daily and multiplied by the percentage of patients presenting that symptom on the same day. This yielded an index of intensity which was considered to provide a more accurate evaluation of symptoms than symptom means. Medication indices were obtained by multiply-

ing the mean intake by the percentage of patients using the drug. Differences between groups were evaluated by Mann-Whitney U test or Fisher's exact test whenever appropriate. Evolution within each group was evaluated by Wilcoxon matched pairs test.

RESULTS

Compliance, tolerance and side effects

All patients completed the study. Daily records from two patients, one in Group 2 and one in Group 3, were incomplete and are not included in the analyses of results. No systemic side effects were noted. In treated patients, the maximum wheal diameter never exceeded 20 mm at the highest dosage level, with the mean diameter being 12 mm. Local pruritus, at the site of injection, was noted by a few Group 1 patients 3 to 6 h after injection.

Symptom scores

Patient self-evaluation. The general subjective benefit resulting from treatment was evaluated using a VAS whose evolution is shown in Fig. 1. During the pollen season (days 45 to 100), the scores for each of the treated groups were significantly lower than for the placebo group (Mann-Whitney U test, $P < 0.01$ in each case). Group 3 patients, who were inoculated with the lowest amount of allergen-antibody complexes, experienced no improvement until day 70, a few days after which the solution containing 120 ng of allergen and 1.2 µg of specific antibodies was administered. As every patient of Group 3 behaved in a similar manner, this suggests that a threshold amount of complexes has to be administered before an effect can be seen.

Nasal scores. Nasal symptoms were recorded on diary cards and an index was calculated as described in Material and Methods by multiplying the mean of symptom scores by the symptom incidence in each group. Fig. 2 charts the evolution of nasal obstruction. During the

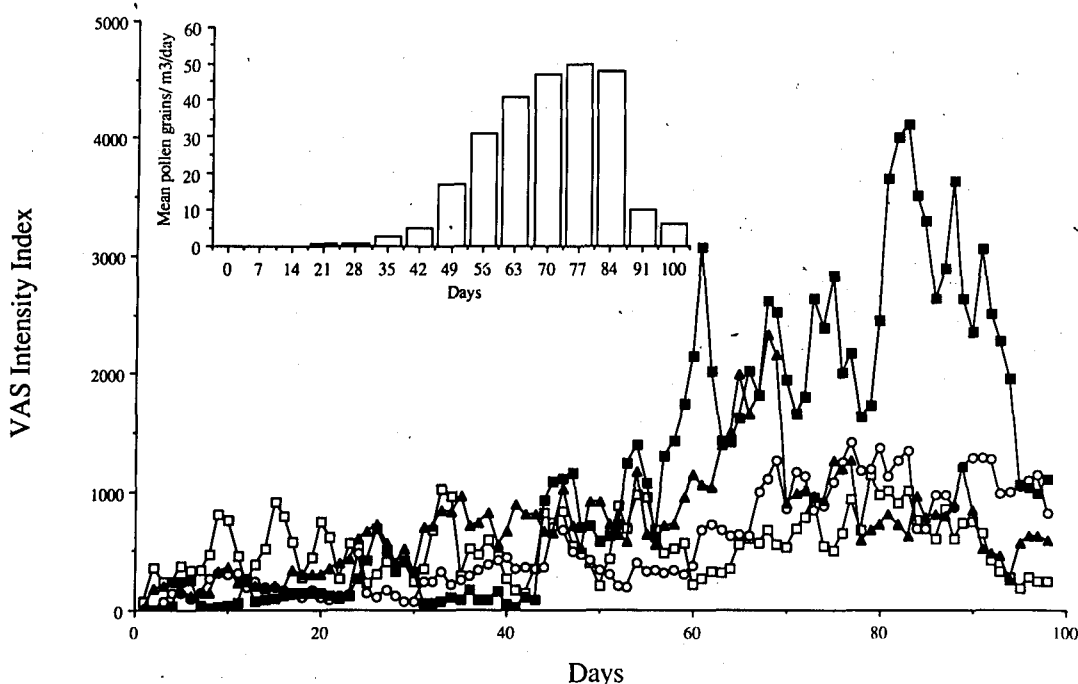


Fig. 1. Evolution of the visual analogue scale (VAS) of Group 1 (□), Group 2 (○), Group 3 (▲) and placebo patients (■). The patients were asked to draw a mark on a horizontal scale graduated from 0 to 100 mm according to their estimation of general well-being. Zero values indicated a very good condition while values of 100 represented very severe symptoms. Intensity indices were obtained by multiplying the mean daily symptom severity score by the percentage of patients presenting that score on the same day. The inset shows the mean pollen counts. A significant number of pollen grains was counted from day 45 onwards.

peak of the pollen season treated groups clearly differed from the placebo group (Mann-Whitney U test, $P < 0.01$ in each case) but no significant difference was seen between treated groups. Symptoms of nasal obstruction in the placebo group were more marked during the second part of the pollen season, at a time when antihistamine intake was lower (Fig. 5). Since some patients complained of nasal obstruction before the pollen season, we calculated the number of patients who experienced a significant increase of symptoms during the pollen season (Table 2). Eleven of 12 Group 3 patients (not shown) had unchanged symptoms except during the early part of the pollen season, at a time when the maintenance dosage had not yet been reached.

Fig. 3 represents sneezing and rhinorrhea combined patterns. Mean indices were significantly lower for patients included in Groups 2

and 3 (Mann-Whitney U test, $P < 0.01$ in each case) than in the placebo group. The number of treated patients complaining of more sneezing and/or rhinorrhea during the pollen season than prior to it was very much reduced compared with the placebo Group (Table 2). In Group 1, half the patients had mild symptoms and the mean index did not differ significantly from the placebo group. Symptoms developed for a few days just after the last two injections and might have been elicited by free allergen in the inoculation solution.

Bronchial asthma. Mean bronchial asthma scores are shown in Fig. 4. Patients in each treated group had significantly lower scores than placebo patients (Mann-Whitney U test, $P < 0.01$ in each case). Table 2 shows that, even when considering very mild asthma (score 1) as significant, only four treated patients out of 37 had

asthma versus seven out of 12 in the placebo group. Two of these four treated patients (both in Group 1) had symptoms clearly related to allergen exposure. The other two patients (both in Group 2) suffered from respiratory tract viral infection which may have exacerbated asthma. None of Group 3 patients had asthma.

Ocular and cutaneous symptoms. The number of patients who experienced either ocular or cutaneous symptoms was too small to allow for a meaningful analysis; evolution of these symptoms during the study are therefore not represented here.

Medication scores

As only the same medications were made available to each patient, groups were compared by computing a drug index. Fig. 5 shows that terfenadine intake (from day 40 to 100) was lower in each of the treated groups compared with the

placebo (Mann-Whitney U test, $P < 0.01$ in each case). No patient in Group 1 or 3 inhaled salbutamol; two patients in Group 2 used the drug, one of whom presenting an acute respiratory tract viral infection. Four patients in the placebo group used salbutamol regularly throughout the pollen season.

In addition, three patients took oral corticosteroids during the peak of the pollen season; they were all in the placebo group.

Specific anti-grass pollen antibodies

Specific IgE and IgG antibodies were assayed after fractionation and adsorption on B2-coupled Sepharose and are expressed in weight/ml serum. Their evolution during the study is represented in Fig. 7. An expected and significant increase of specific IgE antibodies was observed in the placebo group (Wilcoxon matched pairs test, $P < 0.05$), while no significant change was observed in the treated groups.

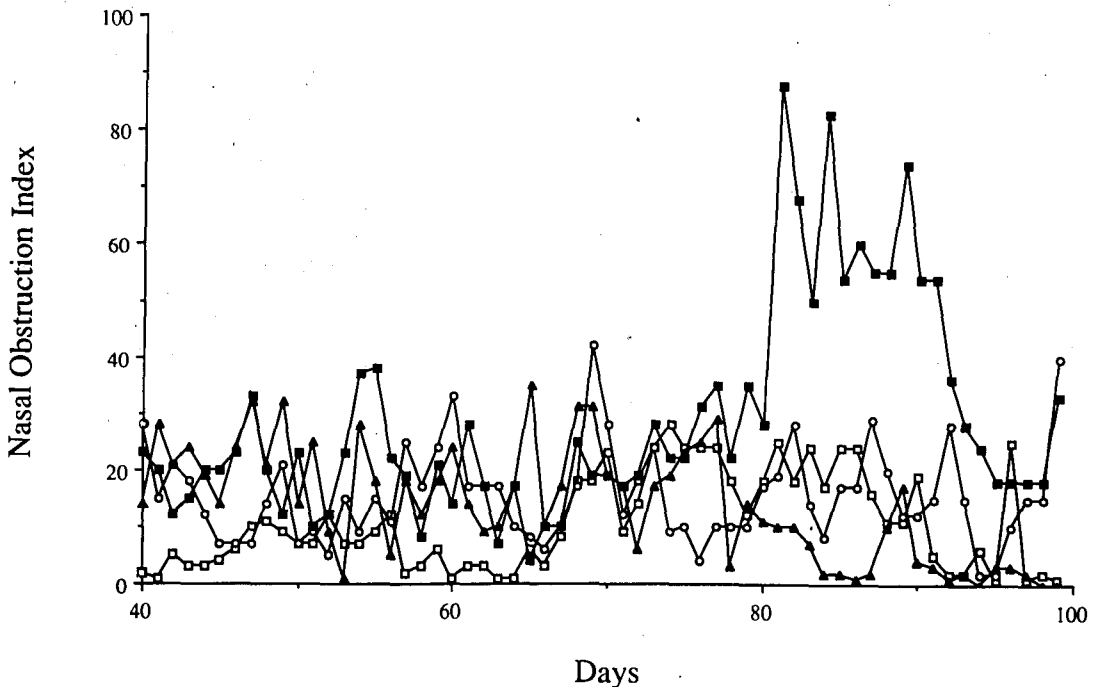


Fig. 2. Evolution of the nasal obstruction index of Group 1 ($\square$), Group 2 ($\circ$), Group 3 ($\triangle$) and placebo patients ($\blacksquare$) during the pollen season.

Table 2.

Comparison of the number of patients whose symptoms appeared or worsened in relation to allergen exposure.

Nasal symptoms were considered as worsened if a change of at least 50% occurred between the mean symptoms score recorded during a week before the pollen season and a week during the peak of the pollen season.

P values were computed by Fisher's exact test

		Treated groups	Placebo	<i>P</i>
Nasal obstruction	unchanged	30	5	0.023
	worsened	7	7	
Sneezing & rhinorrhea	unchanged	29	5	0.029
	worsened	8	7	
Bronchial asthma	none	33	5	0.002
	score ≥ 1	4	7	

Instead, in Group 3, an initial decrease in IgE concentration was observed in 10/12 patients; this reduction did not, however, reach the 5% level of significance when considering the whole group (Wilcoxon matched pairs test, $P = 0.11$) and IgE levels returned to initial values at the end of the trial.

Specific IgG antibodies of placebo patients increased early in the season, while < 5 pollen grains/m³ were counted, and reached a plateau at day 60 (Wilcoxon matched pairs test, $P < 0.05$). No significant changes were observed for any of the treated groups. The evolution of specific anti-B2 antibodies was similar in pa-

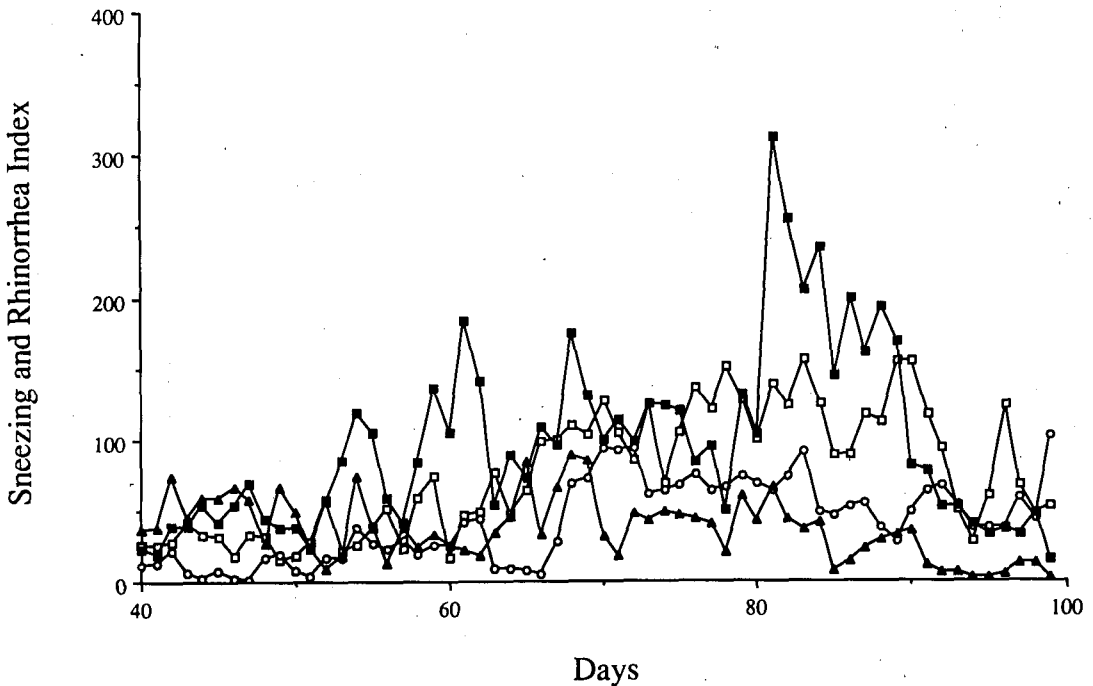


Fig. 3. Evolution of the sneezing and rhinorrhea index of Group 1 ($\square$), Group 2 ($\circ$), Group 3 ($\blacktriangle$) and placebo patients ($\blacksquare$) during the pollen season.

tients who had been hyposensitized by conventional means and in the others; however, formerly hyposensitized patients had a higher specific IgG concentration prior to the pollen season (data not shown).

DISCUSSION

This study evaluates the clinical efficacy and safety of a treatment based on inoculation of allergen-antibody complexes in patients with hay fever and allergic bronchial asthma. It shows that allergen-antibody complex inoculation greatly reduces sneezing and rhinorrhea, eliminates nasal obstruction and bronchial asthma, and does not result in any local or systemic side effects. Seasonal increase in IgE antibody concentrations is prevented without significant boost of specific IgG antibodies.

The grass pollen extract used here is a complex mixture of proteins from 12 of the most commonly encountered pollens of western Europe. Although there exists a wide cross-reactiv-

ity between pollen species, we used it for immunoadsorption to warrant an optimal adsorption of specific antibodies and thereby ensure that all relevant specific antibodies would be present in complexes. This explains the unexpected high yield of specific anti-grass pollen antibodies which can be further ascribed to the immunoadsorption itself; the latter was always performed in a large excess of allergens, a condition which favors the retention of low-affinity antibodies. However, allergen insolubilization prevents access to some epitopes (data not shown) and this can result in the presence of a small proportion of free allergen in solutions used for inoculation.

Allergen-antibody complex inoculation markedly reduced nasal symptoms. Virtually no patient complained of nasal obstruction and only Group 1 patients experienced sneezing and rhinorrhea toward the end of the trial. Since, when this occurred, the symptoms developed within a few hours after injection, we believe that this might be related to uncomplexed aller-

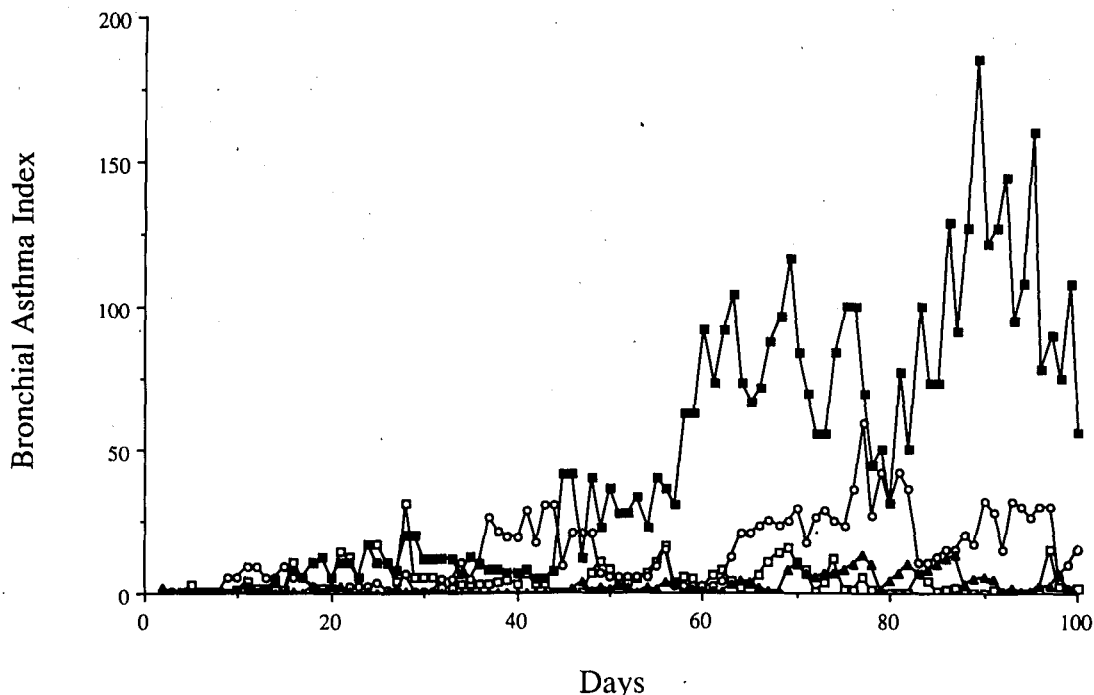


Fig. 4. Evolution of the bronchial asthma index of Group 1 (□), Group 2 (○), Group 3 (▲) and placebo patients (■) before and during the pollen season.

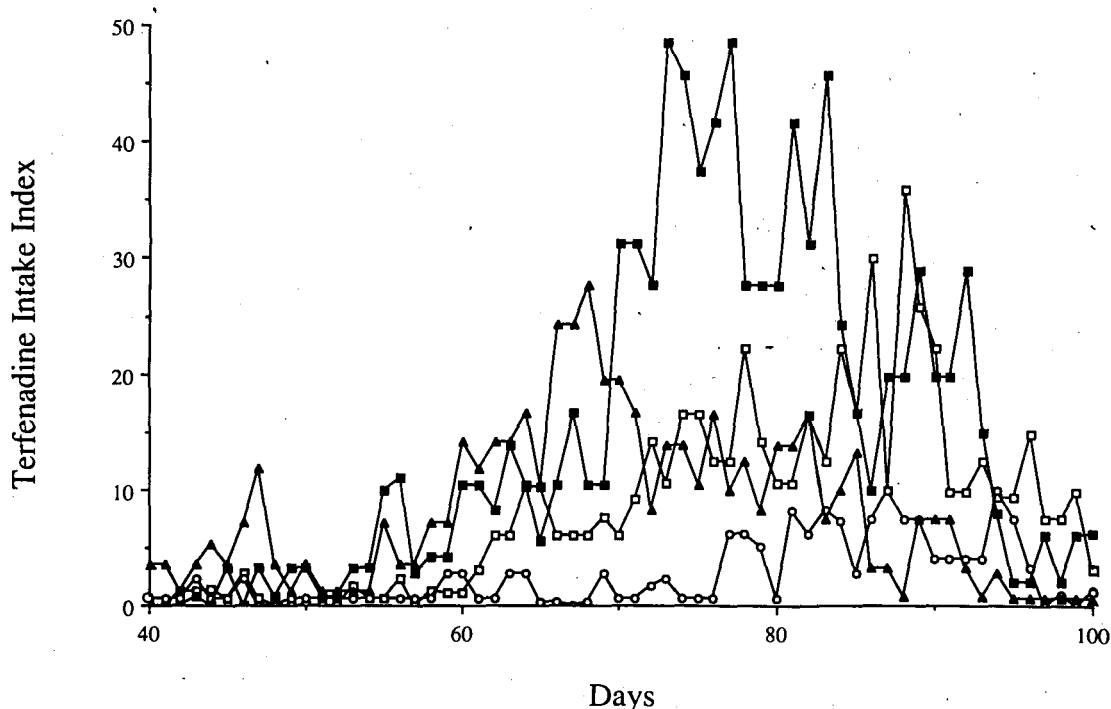


Fig. 5. Intake of antihistamine (terfenadine) by Group 1 (□), Group 2 (○), Group 3 (▲) and placebo patients (■), represented as an index obtained by multiplying the mean daily intake (in number of 60-mg tablets) by the percentage of patients using the drug.

gen in the preparations. In the placebo group, nasal symptoms were more marked during the second half of the pollen season, although antihistamine intake was higher during the first half of the season. This reflected the patient perception of a loss in antihistamine efficacy towards the end of the pollen season, which could be due to inflammatory changes occurring in the nasal mucosa.

The efficacy of the treatment in bronchial asthma appears clear. Only four treated patients suffered from asthma instead of a number of 25 which might have been expected based on the behavior of the placebo group. In two of them presenting an infection of the respiratory tract it was even uncertain whether the asthma was actually elicited by pollen exposure and not by the infection. The amount of allergen-antibody complexes needed for preventing asthma is low as can be seen by the fact that none of Group 3 patients had respiratory symptoms even though no bronchodilator or corticosteroid was taken.

This clinical benefit was observed after inoculation of a cumulative dose of 2.4 μ g specific antibodies and 240 ng allergen. In conventional hyposensitization, an average cumulative amount of 100 μ g, i.e. 400 times more, of the same allergen would be necessary before producing first clinical benefit.

Compared with hyposensitization, which usually requires months of treatment before showing clinical benefit, inoculation of allergen-antibody complexes produces a rapid improvement. In this study, inoculation was started 5 weeks before the expected grass pollen season but this time delay might perhaps be shortened. Our experience with patients hypersensitive to Dpt (unpublished data) has shown that reduction to 4 weeks can be achieved, indicating that the treatment can be started very close to the pollen season. The safety of this approach also enables the treatment to be given during the pollen season itself, as we have done in this study. This yields another advantage over cur-

rent therapy as most pollen-sensitive patients tend to forget about their sensitivity until very late in the season.

We believe this treatment is safe, although our experience is still limited. Absence of systemic side effects and limitation of local reaction to an occasional and moderate local wheal reaction in this study is what we observed in two other clinical trials (16; Machiels et al, 1990a, manuscript submitted). As already discussed, there are at least three reasons which can explain the lack of side effects. Allergen quantities remain small, less than 2 μg /injection at the most (see Table 1). Specific antibodies in excess neutralize allergen epitopes (20) and, finally, intradermal inoculation limits allergen diffusion.

Group 2 patients, who had been successfully treated during the previous pollen season (Machiels et al, 1990a, manuscript submitted), have been included here to determine whether treat-

ment administration over 2 consecutive years may provide a further clinical benefit. As grass pollen air concentrations vary widely from one year to another, it is difficult to compare these patients from year to year. In the present trial, Group 2 patients presented the lowest nasal and medication scores and this was verified for every patient included in this group. A comparison with patients of Group 1 showed that, even though Group 2 patients received the same quantities of allergen-antibody complexes, they did not suffer from sneezing and rhinorrhea. This might indicate that an additional benefit can be gained from a second year treatment. However, the occurrence by chance of the two respiratory infections in Group 2 and the consequent effect on the score of this group prevents any firm conclusion being drawn.

Specific IgE antibodies of treated patients did not increase during the pollen season, which corresponds to what is generally observed after

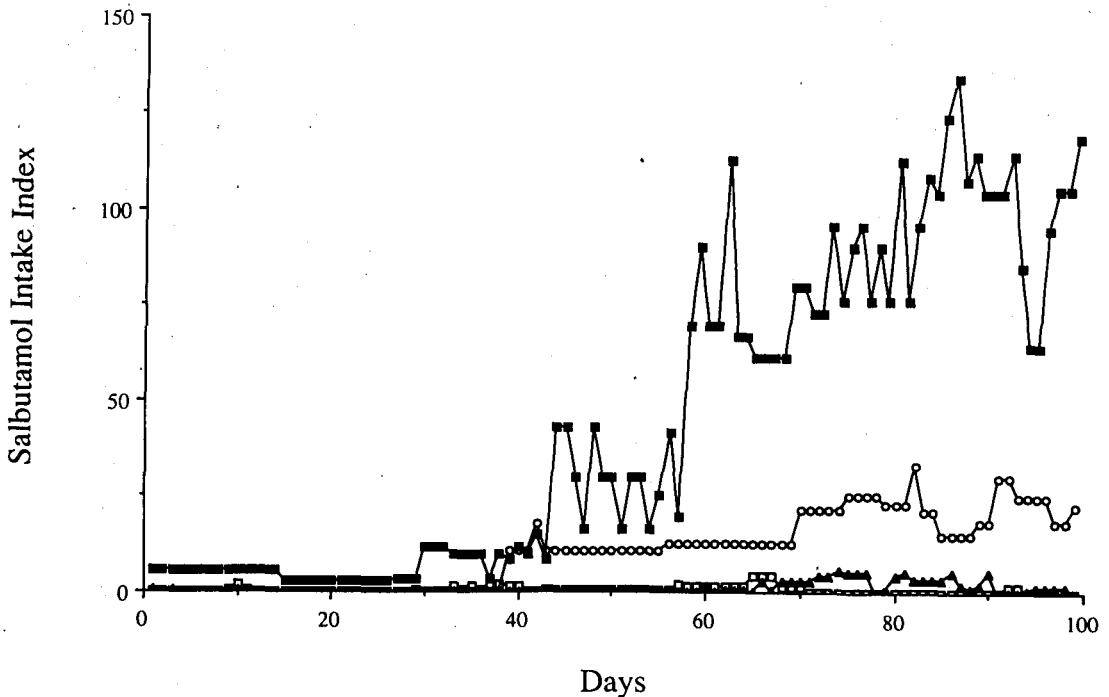


Fig. 6. Intake of bronchodilator (salbutamol) by Group 1 (□), Group 2 (○), Group 3 (▲) and placebo patients (■), represented as an index obtained by multiplying the mean daily intake (in number of puffs) by the percentage of patients using the drug. Erratic drug intake which frequency did not exceed 1/week explains why values for Group 3 patients are not all on the baseline.

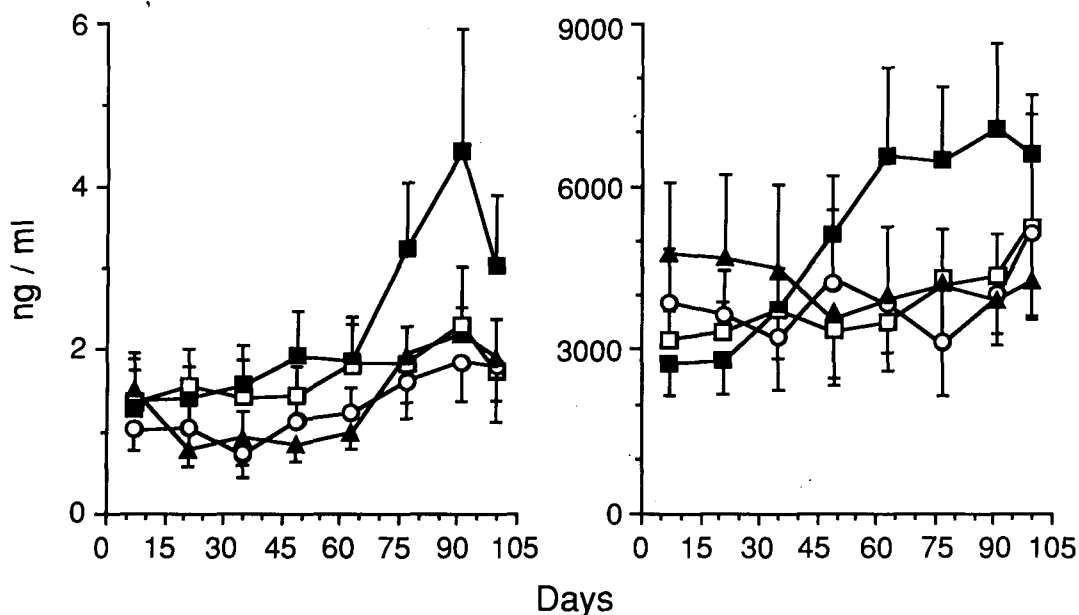


Fig. 7. Evolution of specific anti-pollen IgE (left panel) and IgG (right panel) antibodies in Group 1 (□), Group 2 (○), Group 3 (▲) and placebo patients (■) before and during the pollen season. Vertical bars indicate SEM.

successful conventional hyposensitization. Previous year trial (Machiels et al, 1990a, manuscript submitted) also showed that specific IgE concentrations were lower in the treated patients than in the placebo group and, further, that 3 months after the end of the trial, this reduction was even more profound. In addition to our findings in Dpt-hypersensitive patients (16), where reduction in specific IgE concentration was first seen after the 10th week of treatment, these results suggest that IgE production is reduced as a consequence of the treatment but that tissue-bound IgE antibodies have to be eliminated before significant reduction of serum levels can be seen.

The method used for assay allergen-specific IgE antibodies in this study has been compared with two different serum IgE assays, a latex agglutination assay developed in our laboratory (10) and a solid-phase radioimmunoassay (13) run in an independent laboratory (Prof. N. F. Adkinson, Johns Hopkins University). A good correlation was found between these assays (manuscript in preparation), confirming that al-

lergen-specific IgE antibodies can be reliably assayed after their isolation from the bulk of immunoglobulins (20).

We have compared the evolution of clinical symptoms and specific antibodies of patients who had been treated by conventional hyposensitization with patients who never were. Both evolutions were virtually identical for the two groups. The efficacy of conventional hyposensitization is considered to be dependent on an increase in specific "blocking" IgG antibodies (11). Our findings nevertheless indicate that a significant clinical improvement can be gained without elevation of specific IgG antibodies. Interestingly, untreated patients exhibited a rise of specific IgG antibodies which started while pollen exposure was minimal and, although this increase remained moderate, it correlated with the increase in IgE antibodies. These data are in keeping with the postseasonal boost of anti-timothy grass pollen IgG antibodies shown in hypersensitive children (18). Whether what we observed in treated patients represents active suppression of IgG antibodies induced by anti-

gen-antibody complexes as was suggested from our study of Dpt hypersensitivity (16), or simply the result of using subimmunogenic doses of allergens, cannot be determined at this stage. This question has been addressed during a more recent study in which the effects of allergen-antibody complexes have been compared with that of free allergen at the same concentrations as in the complexes (manuscript in preparation).

Antigen-antibody complexes formed in antibody excess can suppress an immune response (3). Several mechanisms of action have been proposed, such as "blockade" of B cells by large-size complexes (22), induction of anti-idiotypic antibodies (14) or alteration of T-cell response (4). The relevance of these findings to IgE antibody response remains speculative although idiotype suppression of IgE production *in vitro* (9) and *in vivo* (1) has been demonstrated. Whatever the mechanism of action, it appears to differ from conventional hyposensitization in several respects. The clinical potentials of this treatment, together with its presumably novel mode of action, make it worth further investigations both at clinical and experimental levels.

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