

Mediator release is altered in immunotherapy-treated patients: a 4-year study

Dokic D, Nethe A, Kleine-Tebbe J, Kunkel G, Baumgarten CR. Mediator release is altered in immunotherapy-treated patients: a 4-year study. Allergy 1996; 51: 796–803. © Munksgaard 1996.

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Key words: immunotherapy; mediator release; specific IgG antibodies.

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Accepted for publication 16 May 1996

In recent years, it has been possible to demonstrate mediator release into the nasal secretion after nasal allergen challenge in patients with allergic rhinitis. Using the nasal provocation model, we determined whether the mediator release was altered in immunotherapy-treated patients. Seventeen grass-pollen-allergic patients were examined under controlled, reproducible conditions. Serial challenges with increasing doses of grass pollen produced increasing numbers of clinical symptoms and release of mediators such as kinins, TAME-esterase activity, and histamine. Ten patients received a semidepot perennial grass-pollen extract for 4 years. Seven patients served as controls and did not receive immunotherapy during the observation period. Data from the group of patients receiving immunotherapy over the first year already showed a partially significant decline in the maximal mediator release after nasal allergen challenges compared to the results of pretreated challenges, whereas controls did not show any significant changes. Nasal allergen challenges after termination of 4 years' immunotherapy significantly modified the mediator release compared to pretreatment values (TAME-esterase activity $P < 0.05$, kinins $P < 0.01$, and histamine $P < 0.01$). Decrease of mediator release paralleled the symptom-medication scores and quantitative skin prick test. Finally, we could demonstrate a significant correlation between specific IgG increase and mediator decrease in the treated group.

Immunotherapy in patients with pollen allergy has been evaluated by the following criteria:

- 1) clinical parameters (symptom/medication scores)
- 2) rhinomanometry (difficult to assess because of poor reproducibility)
- 3) direct inquiry, which is highly subjective on the part of both the physician and the patient
- 4) the quantitative skin prick test (QST).

Better reproducibility of results was achieved with immunoglobulin measurements (specific IgG and specific IgE), although a statistically significant relation has so far been detectable only between the applied cumulative dose during immunotherapy and the production of IgG antibodies. A direct correlation has not yet been clearly demonstrated between the clinical effectiveness of immunotherapy and IgG antibody production

(1–3). *In vivo* measurement of mediator release in the nasal secretion of allergic patients after nasal allergen challenge now permits an objective evaluation of hyposensitization independently of the subjective parameters mentioned.

In repeated nasal lavages of allergic patients before and after antigen provocation, it is possible to detect the mediators and proteins that trigger the immediate allergic reaction. This applies particularly to histamine, kinins, and TAME-esterase activity. These mediators are detected at the onset of the clinical symptoms (sneezing, secretion). Measurement of these mediators is reproducible to a high degree in allergic patients (4–6).

Therefore, it was the aim of the present study to determine whether mediator release was altered in immunotherapy-treated patients.

Material and methods

Subjects

Seventeen patients (age range: 15–37 years) with grass-pollen allergy of at least a 2-year duration were included in the study. Diagnosis was confirmed by history, skin prick test, and RAST > 2 for grass pollen. None of these patients had previous immunotherapy and asthmatic symptoms, but seven patients had cough symptoms during the pollen season. Concomitant allergy medications were restricted to local cromoglycine applications and oral antihistamines. Ten of these 17 patients received immunotherapy with a 100% standardized grass-pollen extract (NHD, Allergopharma, Germany) over 4 years. Seven patients served as controls over the observation period. Two treated patients dropped out before the study was completed.

A daily symptom/medication score was recorded on a 4-point scale during all grass-pollen seasons and was started one season before the immunotherapy was initiated.

Nasal allergen provocation with following lavage was performed by the method of Naclerio et al. (5). All challenges were performed out of the grass-pollen season, patients being asymptomatic. Briefly, four lavages with 5 ml 0.9% NaCl at room temperature in each nostril were performed to reduce possible resting mediator levels to a stable baseline. Then a diluent provocation followed. Thereafter, increasing dosages of grass-pollen allergen extract 10, 100, and 1000 BU (Scherax, Germany) were given by a glass nebulizer (Deutsche Pharmacia, Freiburg, Germany) which delivers 0.1 ml per puff, in each nostril, followed 10 min later by a 5-ml lavage (0.9% NaCl at room temperature) per nostril. The lavage fluid was immediately put on ice, centrifuged at 3600 g for 10 min at 4°C to remove cellular fragments, and stored in adequate fractions at –70°C until analysis.

The quantitative skin prick test was performed by the method of Dreborg (7). The wheal areas were determined in mm² as an indicator of the skin reaction to prick test solutions: allergen concentrations of 500, 2000, and 8000 BU/ml (Allergopharma, Germany) were used before and after 1 year of immunotherapy and 500, 2000, 8000, and 32 000 BU/ml at the end of immunotherapy. The standard solutions were 0.9% NaCl and 0.1% histamine. All concentrations of the allergen and standard solutions were used in triplicates. All wheal areas greater than 3 mm² were evaluated as positive skin reaction. The concentration (Ch) of allergen eliciting a wheal of the same size as that of 0.1% histamine was estimated by regression line analysis using a log/log model, $\log \text{ wheal area} = a$

(intercept) + b (slope) $\log C$ (concentration of test solution). Ch values were compared in relation to the QST term by the Wilcoxon-Pratt test for connected random samples. The significance level was set at $P < 0.05$.

Serum levels of specific IgG and IgE antibodies were determined before, after 1 year, and at the end of immunotherapy by the commercially available Phadebas IgE RAST (Pharmacia) and by the method of radiolabeled protein A from *Staphylococcus aureus* for specific IgG (8).

Results were expressed as percentage of bound radioactivity for specific IgG and IgE antibodies.

Mediator assays

Histamine determinations. The 0.8-ml samples were mixed with 0.2 ml of 0.8% HClO₄ and centrifuged for 10 min at 1000 g. Histamine in the supernatant was determined by an automated spectrofluorometric assay (9) with a sensitivity of 0.5 ng/ml and a precision of $\pm 5\%$. The intra- and interassay coefficients of variation (SEM/mean $\times 100\%$) were 6% ($n = 7$) and 9% ($n = 6$), respectively.

TAME-esterase activity. Enzymes displaying arginine esterase activity were assayed by the method of Imanari et al. (10), which is based on the liberation of tritiated methanol from the synthetic substrate, ³H-labeled TAME. For nasal lavage procedure, a 40- μ l aliquot of nasal washing and 10 μ l of 0.2 mol/l Tris buffer, pH 8.0, were used. A 10- μ l volume (1.0 by 105 cpm) of ³H-labeled TAME was added, and after 1 h, the reaction was terminated. The sensitivity of the assay is defined as 1500 cpm above background. Both the intra- and interassay coefficients of variation (SEM/mean $\times 100\%$) were 8% ($n = 7$).

Kinin in the nasal secretion was assayed by the method of Proud et al. (6). Briefly, the radioimmunoassay used employed Tyr-0-bradykinin, which was iodinated by the method of Hunter & Greenwood (11). Rabbit antiserum to bradykinin was produced by multiple intradermal injections of bradykinin which had been coupled to chicken serum albumin by the method of Goodfriend et al. (12).

Assays were performed in 0.2 M Tris/0.01 M EDTA/1% lysozyme, pH 6.4, and all dilutions were made in this buffer. A 200- μ l volume of standard bradykinin of unknown preparation, at suitable dilutions, was incubated with 100 μ l of labeled bradykinin (10 000 cpm) and 100 μ l of antiserum (1 : 400 000 final dilution) for 20–24 h at 4°C. In all cases, the antigen-antibody complex was separated from free antigen by polyethylene glycol precipitation.

The assay was capable of detecting a minimum dose of 20 pg brady-kinin/ml. Inter- and intra-assay

coefficients of variation ($\text{SEM}/\text{mean} \times 100\%$) were 3 ($n=7$) and 2% ($n=8$), respectively.

Immunotherapy regimen

Immunotherapy was given as weekly injections starting January 1986 with 5 BU/ml of 100% grass allergen extract (Novo-Helisen-Depot, Allergopharma, Germany), leading up to a maintenance dose of 5000 BU/ml, given at intervals of 4–6 weeks until the end of the study in March 1990. In four cases, a local reaction was noted (induration >5 cm in diameter) at the site of the injection at the beginning of the therapy. Other side-effects of the immunotherapy were not seen during the whole period of 4 years.

Statistical analysis

Wilcoxon's signed rank test for matched pairs was used to compare the allergen-induced mediator release before, during, and after immunotherapy. Across-group analysis (allergic subjects versus control subjects) of allergen-induced increases in mediators was performed nonparametrically with the Mann-Whitney U test. Statistical significance was presumed to be achieved for values of $P < 0.05$.

Results

Clinical response to immunotherapy

The clinical response of the patients, based on their total symptom-medication diary scores for the 4 years of the study, is illustrated in Fig. 1. Scores in year 0 were taken before hyposensitization. A marked reduction of symptoms and drug consumption was observed in the immunotherapy-treated patient group. These findings demonstrate a definite clinical, but partly subjective, improvement in the treated group.

The weekly pollen counts and the total counts for the grass season were remarkably similar in the 4 years under study and also in comparison with the year before treatment, except in year 4. The weekly average from 5 May to 8 August was as follows: year 0: 25.0 pollen counts/ m^3 (100%); year 1: 24.6 (98% of year 0); year 2: 28.9 (115% of year 0); year 3: 22.6 (88% of year 0); and year 4: 16.9 (68% of year 0).

Immunologic response to treatment

It was not possible to demonstrate the expected decrease of specific IgE in the treated group after a 4-year hyposensitization therapy, although a declining tendency was registered. However, specific IgG

significantly increased ($P < 0.05$) with respect to percent binding in the treated group as compared to the controls. In addition, the comparison of the data after 1 and 4 years of hyposensitization therapy with those before therapy revealed significant increases in specific IgG concentrations in the treated group, which were more pronounced after 1 year of therapy (Fig. 2).

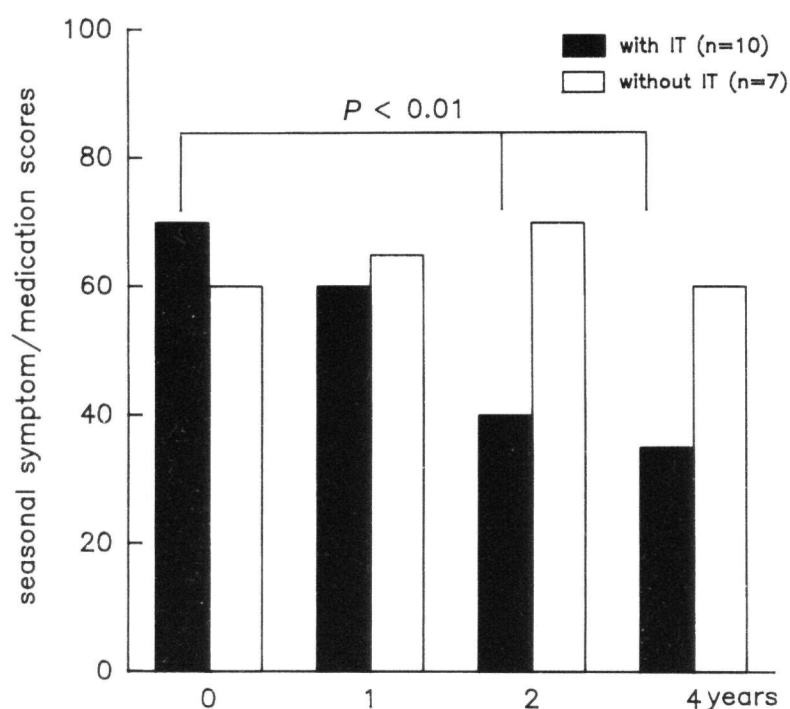


Fig. 1. Symptom-medication diary scores during grass-pollen seasons, beginning with year 0, pollen season in year before initiating hyposensitization treatment (IT = immunotherapy).

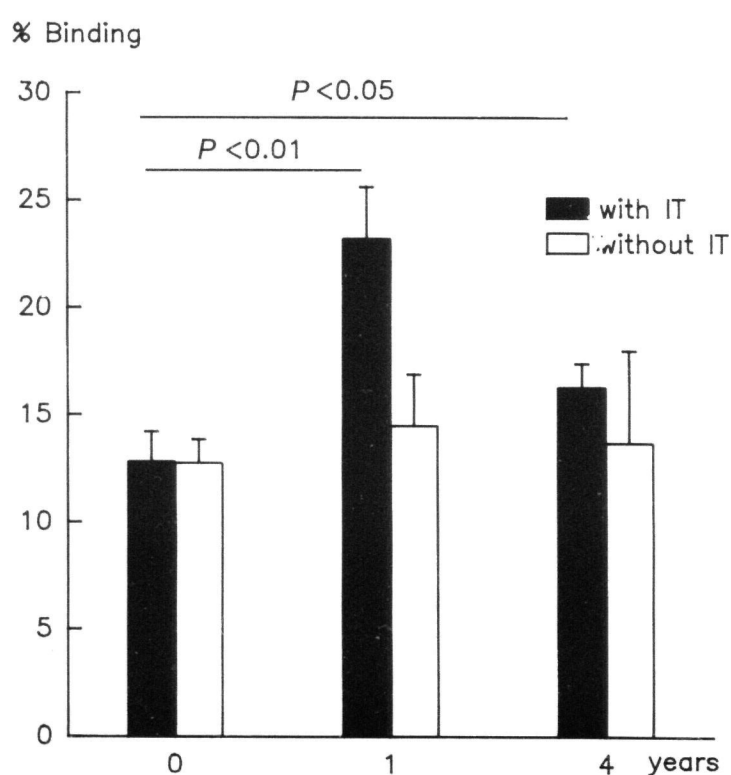


Fig. 2. Concentrations of specific serum IgG antibodies showed significant increases after first year and at end of hyposensitization treatment compared to pretreated levels.

Immunotherapy and skin reactivity

After 4 years of therapy, more than 3.5 times higher concentrations were required (4769 BU/ml versus 1336 BU/ml) to evoke a reaction equivalent to that obtained with 0.1% histamine. After the first year of treatment, the allergen concentration (1906 BU/ml) was nearly 1.5 times the histamine-equivalent dose before therapy (Fig. 3). However, in view of the limited number of subjects ($n=8$) and the partially low values for the 0.1% histamine reaction, these findings should be interpreted with caution.

Immunotherapy and mediator release

A reduction in mediator increase after allergen provocation could be demonstrated. Comparison of TAME-esterase activity before treatment with the activity after 1 and 4 years of therapy revealed a significant decrease 10 min after 200 and 2000 BU antigen and also 20 min after the last antigen challenge (Fig. 4). This was also found for histamine and kinins. For these mediators, the statistical significance was even more pronounced.

Although statistically significant mediator decrease could not be documented for every parameter and every concentration after a year of hyposensitization, there was at least a downward trend for all values.

Fig. 5 illustrates one example of immunotherapy altering mediator responses, showing a decrease in the concentration of the mediators at any dose of nasal challenge after 1 and 4 years of immunotherapy compared to initial levels. In the control

group ($n=7$), no significant changes were seen in the mediator levels after antigen challenge during the whole period of the study.

Furthermore, a significant correlation between the reduction of TAME-esterase activity and of kinin concentration and the increase of specific IgG after 1 year of immunotherapy ($P<0.01$ and $P<0.05$, respectively) could be demonstrated (Fig. 6).

Discussion

The clinical response of the patients on immunotherapy in the presented study was expressed by a significant reduction in patient symptoms and drug consumption during the treatment period. Similar data have been reported by other authors (13). The symptom medication diary score is a subjective parameter in the evaluation of success of immunotherapy since it depends on the pollen count in that particular season, on the awareness of the patients receiving immunotherapy, and placebo effects. To avoid this problem, we compared the symptom medication diary scores from the treated patients with those of the controls. Before initiation of the immunotherapy, both groups had an almost equal median symptom-medication diary score, while after 2 and 4 years of immunotherapy there was a significant difference ($P<0.01$).

Various immunologic changes have been observed in patients receiving immunotherapy, but until now it has not been possible to determine the precise mechanism by which symptoms are reduced in individual cases. What is regularly found after successful immunotherapy is a reduction in the

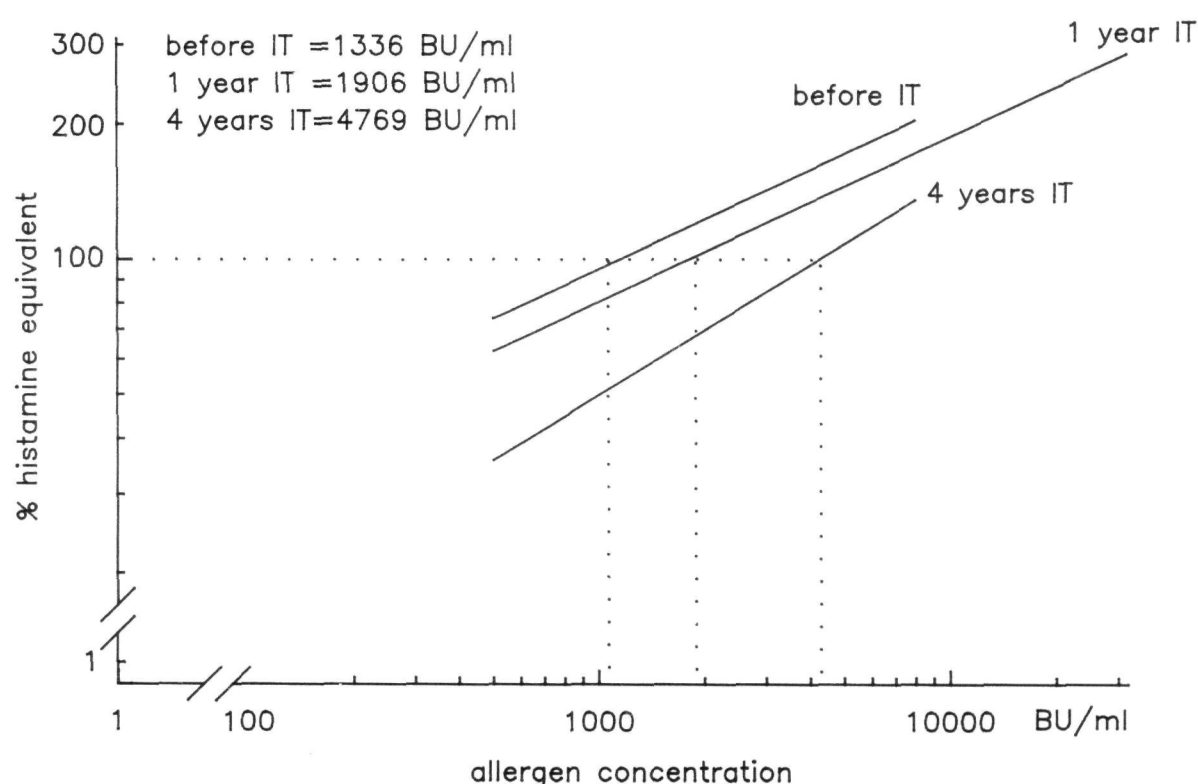


Fig. 3. Comparison of histamine-equivalent skin test results before starting treatment with those after 1 and 4 years of treatment ($n=8$).

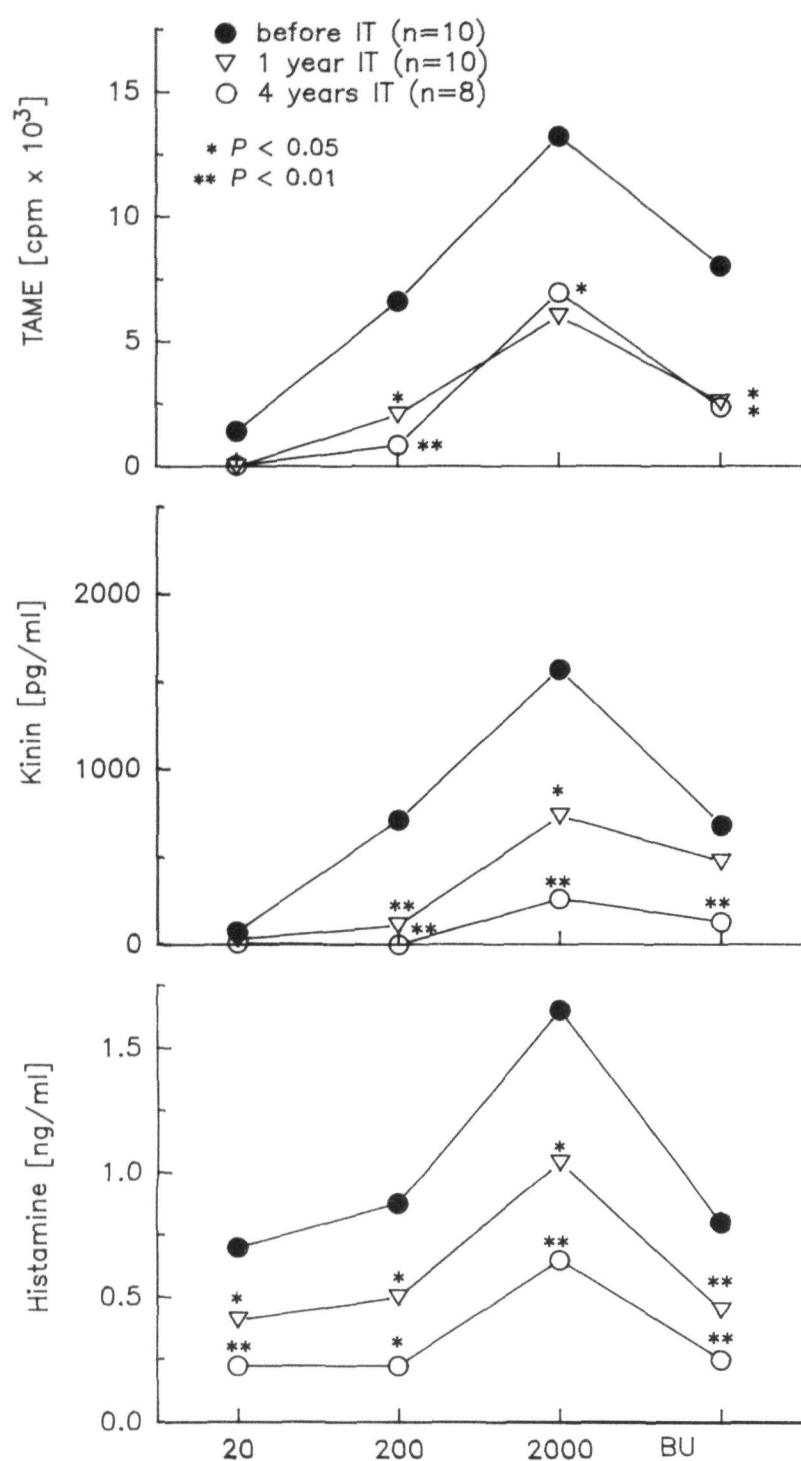


Fig. 4. Dose-response curves of mediators from immunotherapy-treated patients before, 1 year, and 4 years after immunotherapy.

expected seasonal rise in specific IgE antibodies after environmental exposure and a slow decline over several years (14, 15). We also found a decrease, though not significant, of specific IgE levels. This finding suggests a possible connection between immunotherapy and IgE regulation.

Like other immune responses, IgE production is regulated by complex interactions between antigen-presenting cells and T helper (Th) and T suppressor (Ts) cells (16, 17). Atopic patients may have a disturbance of one or more regulatory mechanisms, leading to the tendency to inappropriate IgE responses to various environmental allergens (18, 19).

It has been suggested that selective stimulation of Ts cells or a new formation of antigen-specific Ts cells (20–22) may be responsible for suppressing

IgE synthesis during immunotherapy. In man, isotype- as well as antigen-specific Ts cells have been found in patients treated with immunotherapy, but not in untreated controls (21–23). Successful immunotherapy has been shown to increase numbers of CD8+ T lymphocytes in the skin (24).

The other regular finding in patients receiving immunotherapy is an increase of specific serum IgG antibodies. We could also demonstrate a significant increase of specific IgG antibodies in the treated group as compared to the controls and during the course of immunotherapy. It has been assumed that “blocking” antibodies combine with the allergen at the mucosal surface (25), but this has not yet been proved. Among other possible mechanisms are anti-idiotypic or anti-isotypic regulations (26), or a downregulation by antigen-antibody complexes on IgE-producing B cells via cross-linking of Fc receptors and membrane immunoglobulins (27), thereby reducing their IgE production.

Immunotherapy appears to be specific for the allergen used (28) and in many patients is associated with a specific reduction of the skin sensitivity (29). We found a significant reduction of the QST after 4 years of immunotherapy in the treated group. More than 3.5-fold higher allergen concentration was required after 4 years of therapy to produce the same wheal and flare reaction.

The initial clinical response to nasal challenge with antigen as well as the expected rise in mast-cell-associated mediators is reduced by immunotherapy (30). An influx of inflammatory cells accompanies the late allergic reaction. Both the number of eosinophils and the level of eosinophil cationic protein, an eosinophil-derived mediator, increase during seasonal exposure, and the levels of eosinophils are reduced by immunotherapy (31, 32). Eosinophil cationic protein levels do not increase in patients treated with immunotherapy (33). We could also demonstrate a significant decrease in the concentration of TAME-esterase activity, bradykinin, and histamine in a dose-response relationship in the treated patients with an increase of the applied antigen dose after 1 and 4 years of immunotherapy.

Nasal allergen challenge, which reproduces the symptoms of rhinitis, in inducing pruritus, sneezing, and nasal blockage, is associated with the local release of histamine, prostaglandin D₂, leukotriene (LT) C₄, LTB₄, and tryptase, as identified by analysis of sequential nasal lavages (34, 35). These findings are consistent with mast-cell degranulation. In addition, the kinins kallidin and bradykinin have been identified in nasal lavage fluid after nasal allergen challenge in parallel with symptom generation. Kallidin and bradykinin are vasoactive peptides formed as cleavage products from low- and

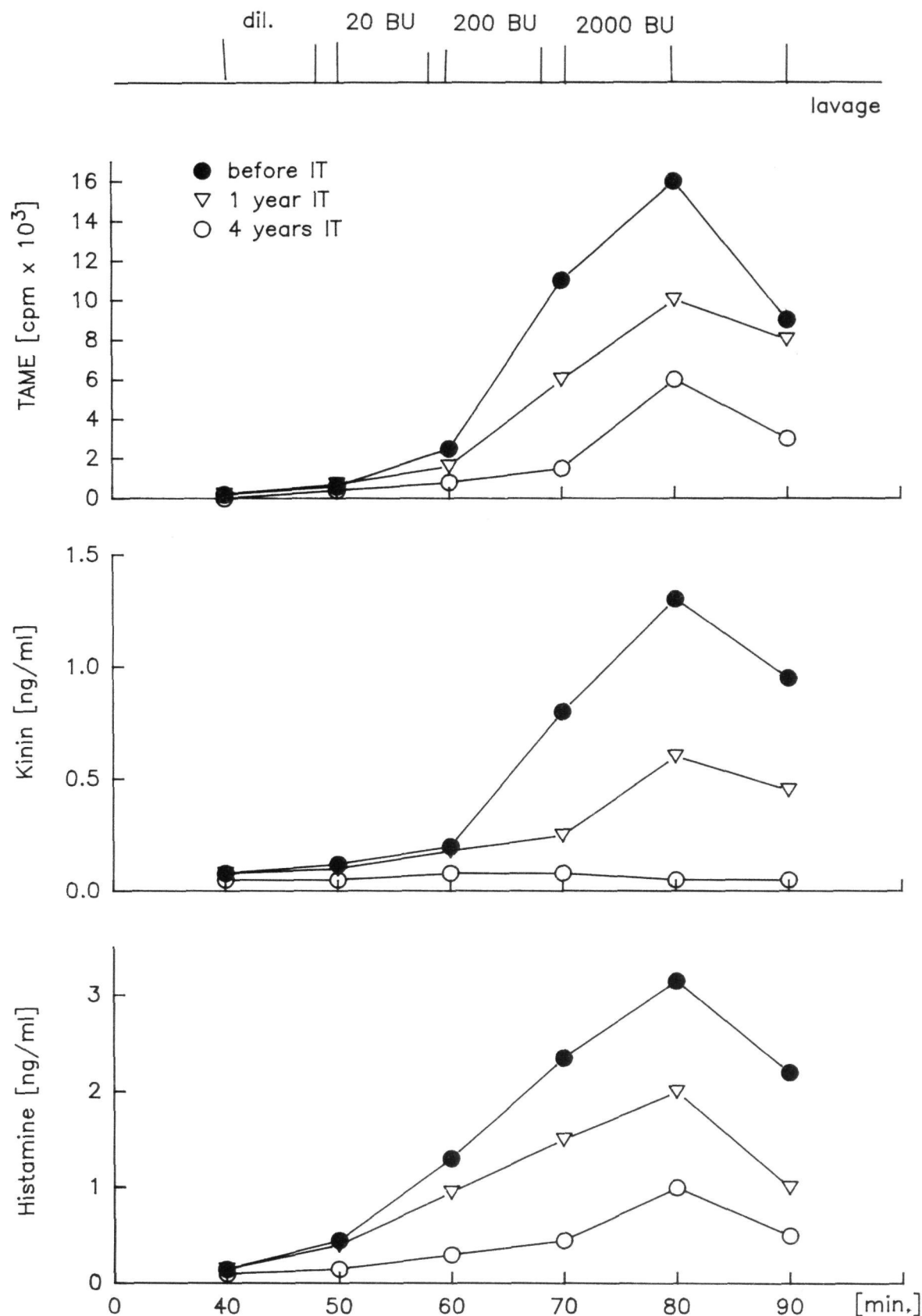


Fig. 5. One example showing that immunotherapy alters mediator response at each dose of applied antigen.

high-molecular-weight kininogens (4) by kallikreins. Because mast-cell tryptase possesses kallikrein-like activity, the local generation of kinins within the nose after nasal allergen challenge can be linked to mast-cell degranulation. TAME-esterase activity in nasal secretion measures mainly a plasma kallikrein- α_2 -macroglobulin complex, together with mast-cell tryptase (36). The described reduction in histamine release by blood basophils (37) and mast-cell numbers, as recently identified in the skin (24), after immunotherapy could lead to a diminu-

tion of the amount of mediators released after allergen challenge.

Antibodies of the IgG and IgA class are induced by immunotherapy and may correlate reasonably well with clinical responses (38, 39), especially in circumstances where the allergen is disseminated via the bloodstream (e.g., Hymenoptera venoms).

Recent observations that passive hyperimmune serum given together with allergen can inhibit the development of adverse reactions during immunotherapy provide compelling evidence that IgG

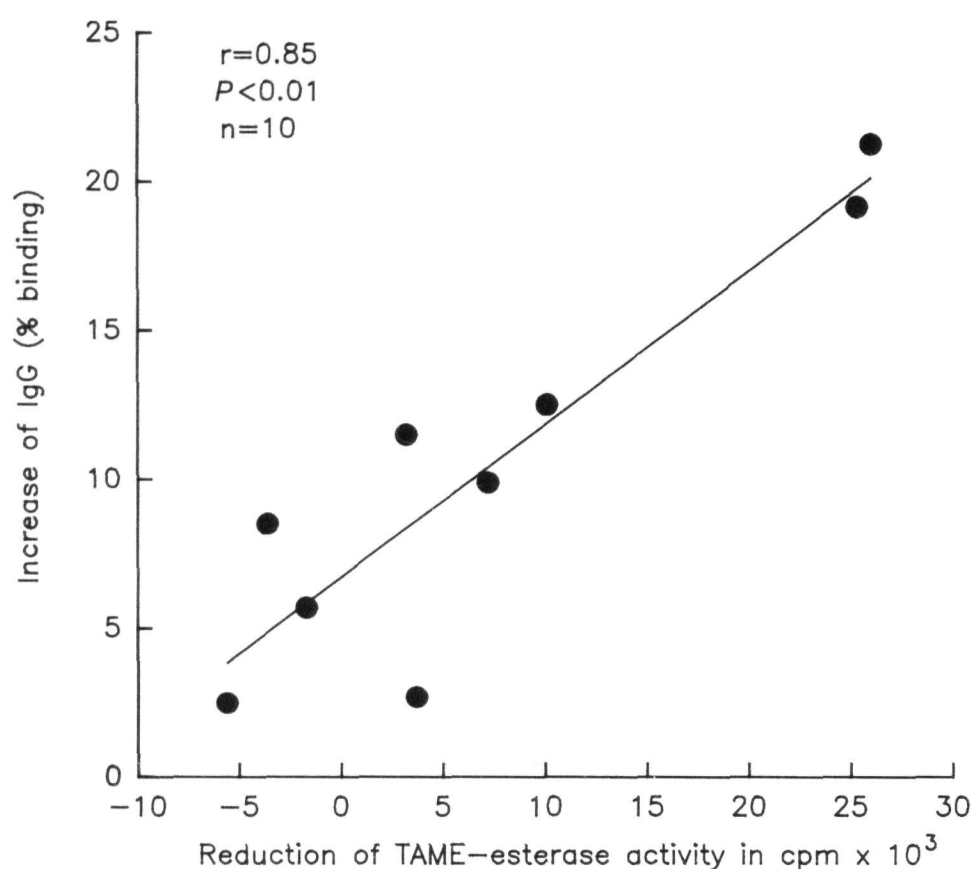


Fig. 6. Correlation between TAME-esterase activity and specific serum IgG antibody after 1 year of immunotherapy.

antibodies can prevent clinical symptoms, at least in the short term (40, 41). While Creticos et al. (13) found no significant correlations between the effect of immunotherapy on nasal mediator release and specific IgG response, Hedlin et al. (1) found a correlation between the decline in TAME-esterase activity and the increase in ragweed IgG and ragweed IgG4 in children. In our study, we could show a significant correlation between the hyposensitization-determined decrease of the TAME-esterase activity and bradykinin concentration and the increase of the specific IgG ($P < 0.01$ and $P < 0.05$, respectively).

Prolonged immunotherapy results in a switch from IgG1 to IgG4 production (28, 42), and the blocking effect of the latter isotype is currently being evaluated. In some patients, there is a lack of complete correlation between allergen-specific IgG titers and clinical response, and it has been suggested that in such cases the IgG response may be directed toward a different epitope or antigen than that of the IgE response (43).

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