

Diagnosis and immunotherapy of mould allergy

VI. IgE-mediated parameters during a one-year placebo-controlled study of immunotherapy with *Cladosporium*

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The IgE-response was evaluated by skin prick test, bronchial provocation test and RAST in a 1-year placebo-controlled double-blind immunotherapy study. Eleven adult asthmatics were treated with a *Cladosporium* allergen preparation and 11 comparable patients received histamine placebo. The bronchial sensitivity (PC₂₀) decrease > 0.5 log step in 8/11 (73%) *Cladosporium*-treated versus 3/11 (27%) in the placebo group. Corresponding figures for skin prick test sensitivity was 10/11 (91%) and 1/11 (9%) respectively. Circulating IgE showed a temporary boost in the *Cladosporium* group and then values approaching the pretreatment value. Only minimal and insignificant changes were found in the placebo-treated patients. Changes in IgE-reactivity were not related to allergen dose, clinical efficacy or to the occurrence of side effects. Some interrelation between changes in skin prick test, bronchial provocation test and RAST was found indicating a differentiated effect of immunotherapy on various IgE compartments. In spite of the pathogenetic role of IgE in allergic diseases, changes in IgE-reactivity do not seem directly involved in the mechanisms underlying the clinical efficacy of immunotherapy but might be of importance in a complex interaction with other immunological parameters.

Key words: bronchial provocation test; *Cladosporium* allergy; clinical efficacy; immunotherapy; RAST; side effects; skin prick test.

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CLINICAL ASPECTS

Immunotherapy has documented clinical efficacy but is both time-consuming and involves side effects. Parameters to predict either the clinical efficacy or the occurrence of side effects would be extremely useful in clinical work. A significantly reduced skin- and bronchial IgE-reactivity was found after 12 months of immunotherapy with *Cladosporium*, but these changes did not have any predictive or estimating implications concerning the clinical outcome. Likewise, neither the pretreatment IgE-reactivity nor changes during immunotherapy was of value as predictors of side effects.

The pathogenetic role of IgE-antibodies in allergic diseases is well established (9). Immunotherapy has a documented clinical efficacy in allergic rhinitis and to a certain degree in asthma (8, 19). Only a few studies on immunotherapy with mould extracts have been published (23), most likely because of the complex nature of mould allergens (26). The clinical efficacy of

Cladosporium immunotherapy has recently been shown by Dreborg et al. in children (5) and by our own group in adults (16). The availability of a highly purified, biochemically, immunologically and biologically standardized and lyophilised extract of *Cladosporium* has made it possible to evaluate changes in IgE-mediated allergic reactions, i.e. skin prick test (SPT), bronchial

cation test (BPT) and circulating IgE (RAST) induced by immunotherapy.

The present study demonstrates immunotherapy-induced changes in SPT, BPT and RAST in 11 adult asthmatics participating in a placebo-controlled double-blind study of *Cladosporium* immunotherapy. Changes in IgE-reactivity are related to those naturally occurring in 11 comparable asthmatics receiving placebo. Further, the immunotherapy-induced changes in IgE-reactivity are related to the clinical efficacy in order to evaluate possible IgE-mediated reactions as predictors of the clinical efficacy and the occurrence of side effects.

MATERIAL AND METHODS

Patients. Twenty-two adult asthmatics with a well-documented allergy to *Cladosporium* completed the first year of the placebo controlled study (11 in the *Cladosporium* group and 11 in the placebo group). Patients in the two groups were comparable with respect to sex, age, duration of mould allergy, associated allergies and clinical severity of asthma disease. Detailed data on the patients participating in the study are given in the paper dealing with the clinical efficacy and side effects of immunotherapy with *Cladosporium* (16).

Allergen extracts. A lyophilised, partially purified and biologically standardized preparation of *Cladosporium herbarum* (Pharmalgen®, Pharmacia, Uppsala, Sweden) was used for immunotherapy and the same batch in all *in vivo* and *in vitro* tests. For *in vivo* tests the extracts were reconstituted on the day of testing in albumin diluent (HSA diluent). The stability of the extract was assured by quantitative immunoelectrophoresis and no degradation in either allergenicity or antigenicity during the observation period was observed. The placebo preparation was caramellized histamine HCl (16).

Study design. Patients fulfilling the inclusion criteria (16) were randomized to either *Cladosporium* or placebo treatment and immunotherapy was initiated in March 1982. SPT was performed before start of immunotherapy (pre-

treatment), immediately before and after the main *Cladosporium* season, i.e. June to October (preseasonal and postseasonal), and after 1 year of treatment. BPT was carried out before treatment, after season and after 1 year. Serum was obtained prior to injections at each cluster of immunotherapy, at maintenance dose (preseasonally), after season and at termination of the placebo-controlled study. Serum was stored in small aliquots at -20°C until analysed and only frozen and thawed once.

Immunotherapy. A clustered regimen was used during the dose-increase phase with four scheduled visits, each with five injections given every fortnight. Maintenance dose was administered once monthly with a modest reduction during the mould season. The dose was reduced accordingly in the case of systemic and immediate local reaction exceeding 5 cm (16).

Skin prick test (SPT) was performed as previously described (14). In short, quadruplicate tests with five 10-fold dilutions of *Cladosporium* (10–100,000 BU/ml) were performed by the same investigator throughout the study. Skin sensitivity (SSH) was calculated as the allergen concentration eliciting a wheal equal to that induced by histamine HCl 1 mg/ml after performing linear regression of the dose-response curve. Tests with a dose-response curve based on less than three 10-fold dilutions, a coefficient of correlation < 0.9 , and a reproducibility (SD %) of the quadruplicate test $> 40\%$, were discharged as non-valid and repeated. Anti-allergic medication interfering with SPT was avoided at least 72 h prior to SPT (14).

Bronchial provocation test (BPT) was carried out according to the technique described in a previous paper (15). One ml of aerosolized extract was inhaled for 5 min every 15 min until a decrease in FEV_1 of $> 20\%$ of the baseline value obtained with HSA diluent. Each BPT was performed with concentrations from 1–100,000 BU/ml. Interruption of the provocation test before inhalation of 1 ml resulted in correction of the BPT results accordingly. Bronchial sensitivity was calculated as the allergen

concentration eliciting a decrease of 20% in FEV_1 (PC_{20}). The reproducibility of repeated tests was within 0.5 log step. Drug treatment interfering with the immediate bronchial response was discontinued as previously described (15).

Specific IgE-determinations (RAST): RAST was performed by coupling *Cladosporium* allergen preparation in a concentration of 100,000 BU/ml to CNBr-activated paper discs. Duplicate samples were incubated overnight at room temperature with undiluted, 5- and 25-fold diluted serum. Second incubation was with ^{125}I -labelled affinity purified rabbit anti-human IgE (Phadebas®, RAST isotop). The assay was calibrated in relation to a timothy-allergic serum and expressed in SU/ml undiluted serum. Dilution of serum allowed the specific binding to be read at the linear part of the dose-response curve. The coefficient of variation of double determinations was less than 10% (15).

Clinical efficacy was expressed semiquantitatively as changes in symptom score (asthma + peak flow score) and medication score registered in the peak *Cladosporium* season during corresponding 10-week periods in 1981 (pretreatment) and 1982 (after 5–7 months of treatment). Symptom and medication scores were defined ("improved", "unchanged" or "deteriorated") as evident from the paper dealing with the clinical efficacy of immunotherapy (16). Thus, the overall clinical efficacy was scored from +2 (improvement of both symptom and medication scores) to -2 (deterioration of both scores).

Statistics. Differences between groups were evaluated by Mann-Whitney's U-test and a Chi-square test. Correlations were calculated by Spearman's Rho.

RESULTS

Pretreatment assessment. Randomization was based on SPT- and BPT-sensitivity, which was not statistically significantly different between groups; neither was the pretreatment severity of asthma disease (16).

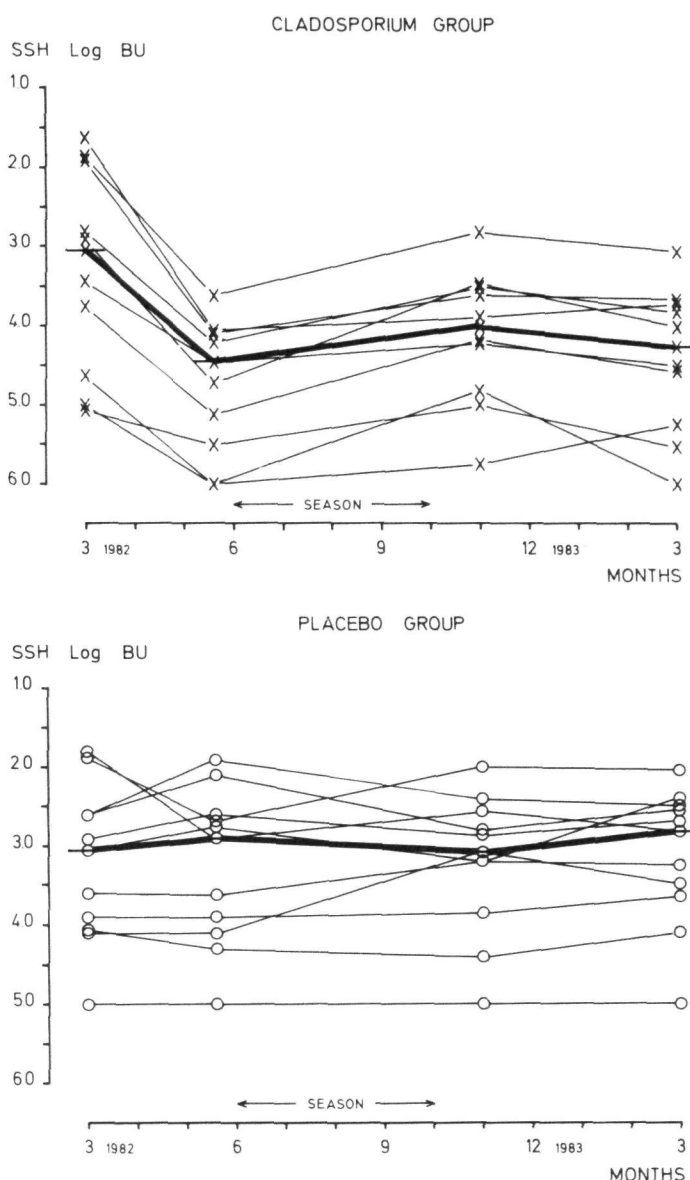


Fig. 1. Changes in skin prick test sensitivity estimated as the allergen concentration eliciting a wheal equal in size to histamine HCl 1 mg/ml (SSH). The heavy line represents median values.

Cladosporium group (×); placebo group (○)

Clinical efficacy. A statistically significant difference in favour of *Cladosporium* treatment was found in terms of clinical efficacy (16). Reduced/unchanged symptoms were seen in 81% *Cladosporium*-treated contrary to 27% in the placebo group ($P = 0.01$).

SPT-sensitivity during immunotherapy. Changes in skin sensitivity in the *Cladosporium* and placebo group respectively are shown in Fig. 1. In the *Cladosporium* group a significant ($P < 0.01$) reduction in skin sensitivity from pretreatment to pre-season was observed, amounting to 1.5 log step. During the season the skin sensitivity

increased ($P < 0.01$) almost 0.5 log step; from preseason to 1 year of treatment, another reduction was registered ($P < 0.05$). The total reduction during the study ranges from 0.26 to 2.06 log step (median 1.2 ($P < 0.01$)).

In the placebo group only minimal and non-significant changes in skin sensitivity were observed (Fig. 1). Contrary to the *Cladosporium* group, no consistent change occurred during the season. The overall changes range between a reduced skin sensitivity of 1.01 log step and an increase of 1.2. The median value was an increase of 0.08 log step (i.e. a slight deterioration in the SPT-estimated IgE-reactivity).

As mentioned, no significant difference in pre-treatment skin sensitivity between the two groups was found. At preseason the difference was highly significant ($P < 0.01$) with a median difference of 1.5 log step. Postseasonally and at 1-year treatment, P -values were < 0.02 and < 0.01 respectively with median differences of 0.75 and 1.5 log step.

Changes in skin sensitivity in both groups were a consequence of a parallel shift of the dose-response curves. The slope of the dose-response curve of all four test series did not change significantly.

Bronchial provocation test sensitivity during immunotherapy. Individual values of BPT sensitivity (PC_{20}) estimated before treatment, before season and after 1 year of treatment are evident from Fig. 2. As regards the *Cladosporium* group, changes observed during the dose-increase phase were not significant. From season to 1 year of treatment, the median PC_{20} was increased by more than 0.5 log step ($P < 0.01$). In the placebo group some fluctuations were registered but

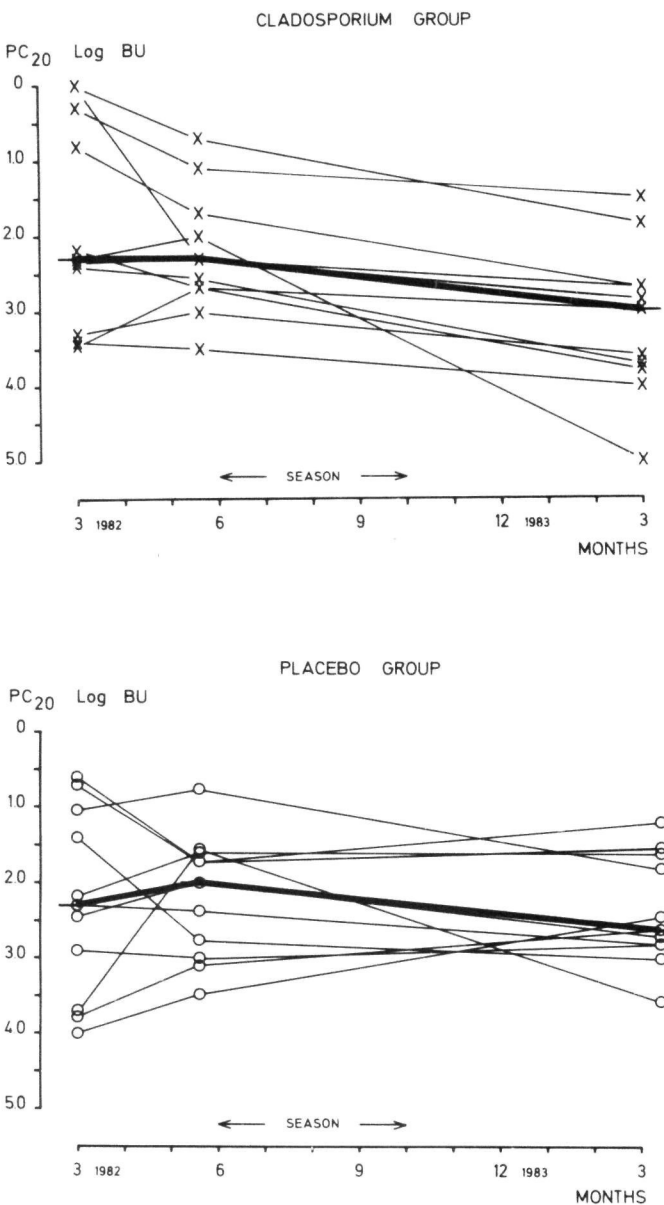


Fig. 2. Changes in bronchial provocation test sensitivity estimated as the allergen concentration eliciting a decrease in FEV₁ of 20 % (PC_{20}). Median values are indicated by heavy lines. *Cladosporium* group (x); placebo group (o)

Table 1
Changes in BPT- and SPT-sensitivity exceeding 0.5 log step evaluated after 1 year of treatment

	Treatment	Reduced	Unchanged	Increased	P-value
BPT	<i>Cladosporium</i>	8	3	0	0.05
	Placebo	3	5	3	
SPT	<i>Cladosporium</i>	10	1	0	< 0.001
	Placebo	1	7	3	

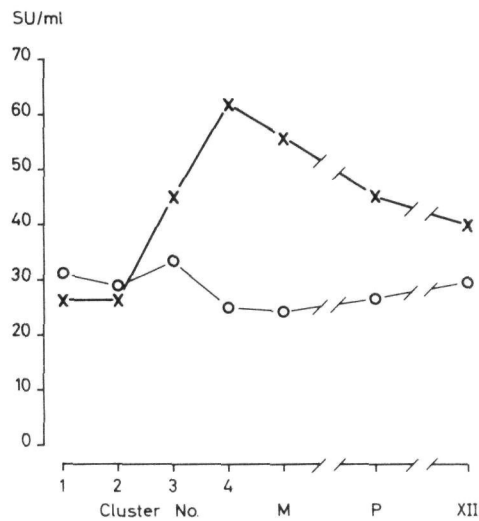


Fig. 3. Median values of circulating specific IgE (RAST) in *Cladosporium* group (x) and placebo group (O). RAST was performed at each cluster during the dose-increase phase, at maintenance dose = preseason (M), at postseason (P), and after 12 months of treatment (XII).

changes did not reach significant levels. The dissociation between PC₂₀-values in the two groups reached a statistically significant level ($P < 0.02$) after 1 year of treatment.

Considering exclusively the changes in BPT-sensitivity exceeding 0.5 log step, a reduced sensitivity was observed in 73 % of *Cladosporium*-treated versus 27 % placebo-treated. An increased BPT-sensitivity was found in 0 % *Cladosporium*-treated versus 27 % in the placebo-group ($P = 0.05$) (Table 1). Correspondingly, reduced SPT-sensitivity was found in 91 % in the *Cladosporium* group and in 9 % placebo-treated. Increased SPT-sensitivity was 0 and 27 % respectively ($P < 0.001$) (Table 1).

Specific IgE (RAST) during immunotherapy. Median RAST-values are shown in Fig. 3. In *Cladosporium*-treated the first cluster did not cause any changes but clusters 2 and 3 resulted in more than a doubling of the median IgE-value ($P < 0.01$). From cluster 4, a significant ($P < 0.01$), steady decrease of IgE was observed, but only three patients (27 %) reached values below the initial level. The 1-year-value, however, was not significantly different from the pretreatment value. In the placebo group only minimal and non-significant changes were

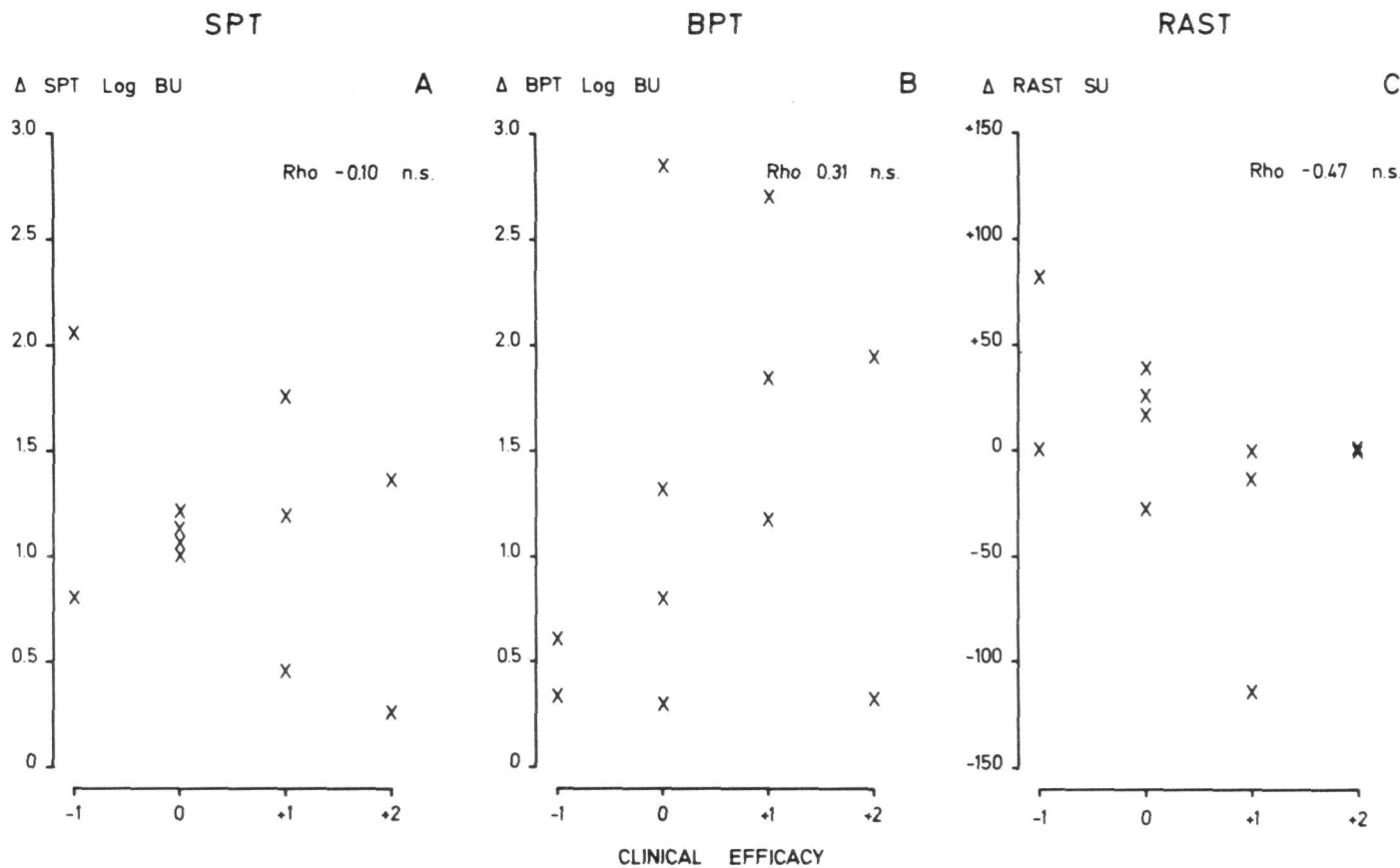


Fig. 4. Relation between changes of SPT, BPT and RAST after 12 months of immunotherapy and the clinical efficacy. For detailed information of the evaluation of clinical efficacy, see text.

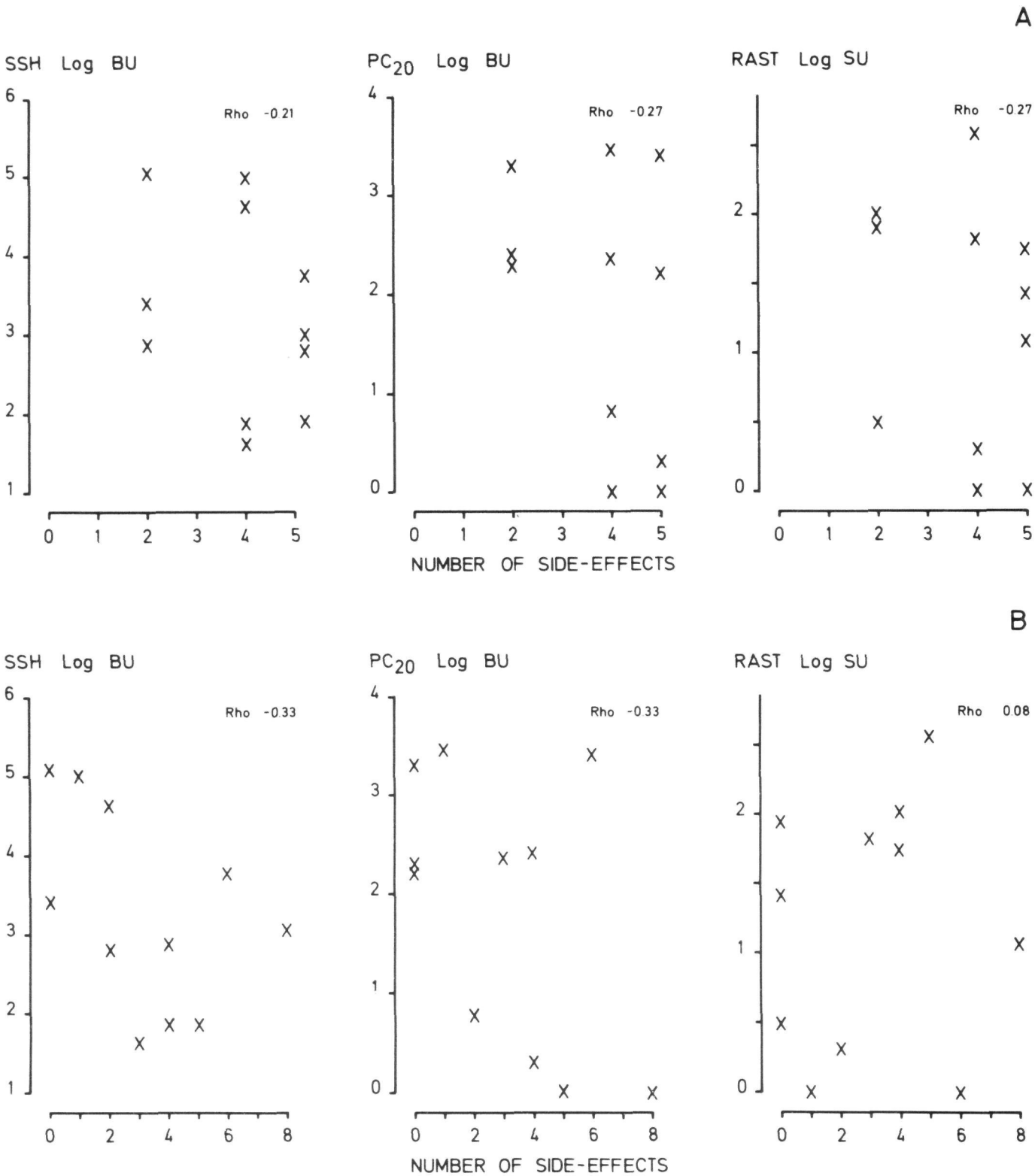


Fig. 5. Relation between pretreatment degree of SPT-, BPT- and RAST-sensitivity and the occurrence of local (5A) and systemic (5B) side effects.

observed. Due to the wide individual variation in specific IgE, no significant difference between the two groups was seen. In the placebo group, three patients had increasing IgE-values during the season, whereas two patients had decreased.

SPT, BPT, RAST and allergen doses during immunotherapy. Changes during the first year of immunotherapy in SPT, BPT and RAST in *Cladosporium*-treated patients were not significantly correlated to the cumulative dose of allergen (Rho -0.24, -0.60 and -0.10 for SPT,

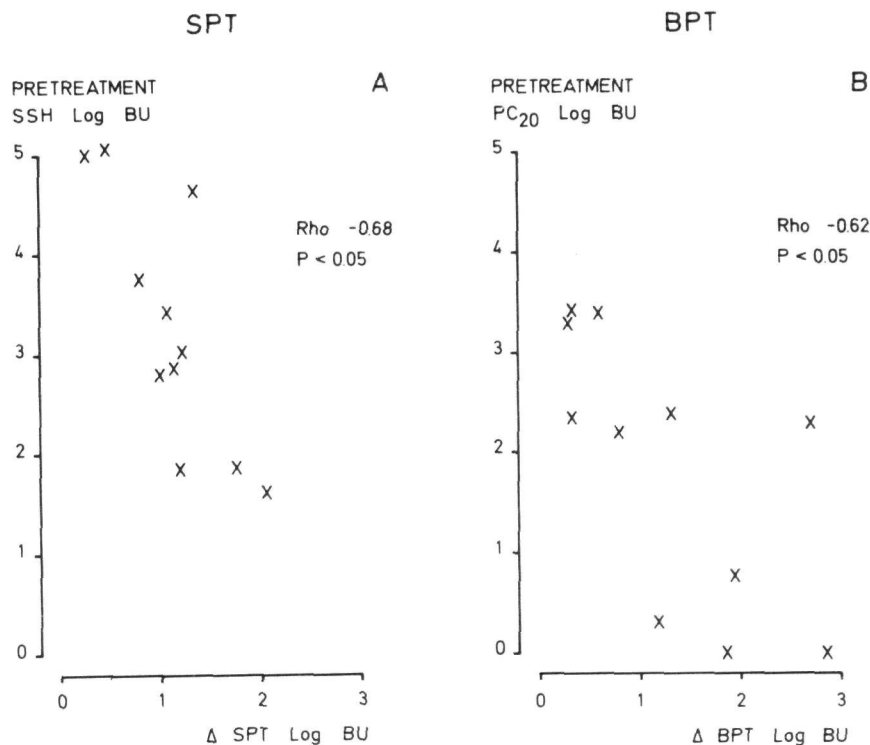


Fig. 6. Relation between pretreatment degree of IgE-sensitivity estimated by SPT (6A) and BPT (6B) and total changes of 12 months of immunotherapy.

BPT and RAST respectively). A significant correlation could not be found between either pretreatment degree of SPT, BPT and RAST sensitivity and maximum top dose (Rho 0.33, 0.49 and -0.08) or maintenance dose (Rho -0.21 , 0.16 and 0.33).

SPT, BPT, RAST and clinical efficacy. The relation between clinical efficacy of immunotherapy and the total changes of SPT-, BPT-, and RAST-sensitivity after 1 year of treatment, did not reach statistically significant levels (Rho -0.10 , 0.31, -0.47 respectively) (Fig. 4). SPT-, BPT-, and RAST-change in dose-increase phase of immunotherapy (from pretreatment to preseason) was not significantly correlated to the clinical efficacy (Rho -0.28 , 0.11 and -0.27). Likewise, pretreatment degree of SPT-, BPT- and RAST-sensitivity was not correlated to the clinical efficacy (Rho 0.01, -0.17 , -0.18).

SPT, BPT, RAST and side effects. Side effects did not seem related to the pretreatment estimation of SPT-, BPT- and RAST-sensitivity. Figures for local side effects were Rho -0.21 , -0.27 ,

-0.27 and systemic side effects Rho -0.33 , -0.33 and 0.08 respectively (Fig. 5).

Interrelation between SPT, BPT, and RAST. The relation between changes of SPT and BPT from pretreatment to preseason and from pretreatment to 1 year was not significant (Rho 0.52 and 0.21 respectively). The corresponding relation between SPT and RAST was Rho 0.70 ($P < 0.05$) and 0.20 respectively. BPT and RAST were not significantly correlated (Rho 0.08 and 0.05 respectively). A certain relation was found concerning absolute values of SPT versus BPT. Rho -values of -0.30 (NS) were found for pretreatment values, Rho 0.30 (NS) at preseason and Rho 0.68 ($P < 0.05$) after 1 year of treatment. SPT versus RAST was significantly correlated before treatment (Rho -0.73 ($P < 0.02$)) and at preseason (Rho -0.70 ($P < 0.05$)) but not after 1 year (Rho -0.49 , NS). BPT and RAST were not correlated (Rho -0.33 , -0.19 and -0.57 respectively).

The relation between pretreatment sensitivity and changes after 1 year of immunotherapy was significant for SPT (Rho -0.68 ,

$P < 0.05$) and for BPT (Rho -0.73 , $P < 0.02$) but not for RAST (Rho -0.12 , NS) (Fig. 6).

DISCUSSION

A fundamental problem in the evaluation of allergen-specific immunotherapy is the mechanisms involved in the clinical efficacy. Several theories primarily based on the animal model have been published. These mechanisms involve a reduction in the pathogenetic IgE, evolution of antigen-specific IgG, reduced mast cell releasability, reduced sensitivity to allergic mediators, etc. (6, 9, 11).

From a clinical point of view the exact sub-cellular mechanisms underlying the efficacy of immunotherapy might not be the most important, whereas establishment of test procedures predictive of both the clinical outcome and the occurrence of side effects would be extremely useful in order to either cancel the treatment or make necessary correction.

Changes in IgE-reactivity during immunotherapy. The present study showed a significant reduction in skin sensitivity, with the median tolerance increased by more than a factor 10. Such changes are reported in a number of studies (2, 3, 4, 8, 20, 27, 29). In most studies, however, the criteria for SPT are not as strict as in the present study (14), and data based on testing with a single concentration are not reliable. As in other placebo-controlled studies, the skin sensitivity in the placebo group showed remarkably small oscillations (3, 5, 17, 27). As late-phase skin reactions were extremely unusual, a possible suppression of the late phase could not be evaluated (21).

As the lungs are the target organ in asthma, an immunotherapy-induced reduction of the bronchial sensitivity might be beneficial. Using carefully performed titrated bronchial challenge tests, we found a significantly reduced immediate sensitivity in the *Cladosporium* group after 1 year of treatment and no changes in the placebo group. The median tolerable dose was increased by more than a factor 5, data comparable with findings in other studies (3, 5, 7, 18, 25, 27).

Two recent studies, however, did not show an increased bronchial threshold (22, 28), except for the late reaction (22). Late reactions, however, were the exception in the present study, possibly a consequence of the intense medical treatment of the immediate reaction.

The circulating specific IgE-response was similar to that published in other studies (8, 13), i.e. a remarkable increase after two clusters with a fortnight's interval (13) and then decreasing values, not statistically different from the pretreatment value. The very limited duration of the IgE boost might explain the discrepancy with several studies that show no changes in IgE (3, 24). In the paediatric *Cladosporium* immunotherapy study (10), a steady decrease from pretreatment value was observed, indicating a possible, age-related difference in the immune response. Based on the placebo group neither the present nor the paediatric study (10) has been able to demonstrate a seasonal increase in specific IgE observed in pollen hay fever (12, 20).

IgE-reactivity and allergen dosage. IgE-reactivity might be considered a determinant of the maximally tolerated allergen dose (5). However, as earlier published in Hymenoptera immunotherapy (13), no correlation between pretreatment IgE-reactivity and allergen dose exists, indicating that factors other than the IgE-reactivity determine the tolerance of allergen administered during immunotherapy.

Evaluating the relation between allergen dose and changes in immunological parameters, Dreborg et al. (5) found a significant correlation between allergen dose and changes in skin sensitivity but not in bronchial sensitivity. The present study revealed no significant relationship, although dose versus BPT approaches significant levels.

IgE-reactivity and clinical efficacy. Neither the pretreatment IgE-reactivity nor the changes were related to clinical efficacy and therefore of no predictive value. A major implication of these findings is that changes in BPT cannot be used as a parameter of clinical efficacy as claimed by Aas (1), at least not in mould allergy immuno-

therapy. Furthermore, doubt is thrown on the validity of several studies published on the efficacy of immunotherapy in asthma (17). A single study using grass pollen has shown a positive relation between symptoms in the season and SPT threshold changes after 12 weeks of treatment (29). Other immunotherapy studies, however, do not show any predictive value of IgE-reactivity (17, 21). It appears that when no relation exists between changes in IgE-reactivity and clinical efficacy, other mechanisms than the exclusively IgE-mediated, must be involved in the immunological mechanisms of immunotherapy. Or, what is more reasonable, the overall efficacy is a complex interaction of various mechanisms in the individual patient (19).

IgE-reactivity and side effects. One would expect to find a rather close relationship between the degree of allergy estimated by the IgE-reactivity and the occurrence of side effects. The problem, however, has not been studied intensively, although some relation between systemic side effects and pretreatment nasal provocation test has been published (30). Studies on Hymenoptera immunotherapy by our own group did not show any relation between pretreatment IgE-reactivity and side effects (13).

Interaction of IgE-reactivity. In spite of the change in IgE-reactivity in SPT, BPT, and RAST – parameters expressing the degree of IgE-sensitization – they seem only partly interrelated. A significant correlation was found between total changes in SPT and RAST and the absolute values of SPT and BPT after 1 year of treatment and SPT and RAST pretreatment and preseason. These data point to a differentiated effect on various IgE-compartments of immunotherapy, probably caused by various factors regulating either circulating IgE or the releasability of mast cell bound IgE, alternatively the response to allergic mediators in the shock organ (6).

Conclusion. Immunotherapy initiates specific and significant changes in IgE-reactivity estimated by SPT, BPT, and RAST. These changes,

however, do not seem related to either the clinical efficacy or side effects of immunotherapy. These parameters therefore cannot be used as a predictor or as an estimate of clinical efficacy. Subsequently, it may be questioned whether the IgE-reactivity is directly involved in the mechanisms underlying immunotherapy or takes part in a complex interaction with other parameters such as IgG. Data on the relation between clinical efficacy and IgE-reactivity and the IgG-subclass response estimated by RAST and IgG-CRIE are in progress.

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