

Diagnosis and immunotherapy of mould allergy

VIII. Qualitative and quantitative estimation of IgE in *Cladosporium* immunotherapy

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The interrelation of *in vitro* IgE-mediated parameters, i.e. serum-specific IgE (RAST), basophil cell-bound specific IgE, and histamine release from basophil leucocytes was investigated in a 1-year placebo-controlled, double-blind *Cladosporium* immunotherapy study involving 22 adult asthmatics. The intense and early burst (within 6 weeks of immunotherapy) of serum-specific IgE did not result in a corresponding increased binding of specific IgE molecules to basophils. Cell-bound IgE increased in the *Cladosporium* season in both groups at the same time as serum levels of specific IgE declined in the *Cladosporium* group. In the placebo group histamine release from circulating basophils paralleled changes in basophil-bound IgE. In *Cladosporium*-treated patients, histamine release cell sensitivity after a lag phase (during immunotherapy dose-increase) declined two log steps, i.e. the cells became less responding in spite of a significant increase in cell-bound IgE. To further evaluate the sensitizing capacity of circulating specific IgE, passive sensitization studies were performed using basophils from a single donor. Although sera taken at the maximal IgE-response showed an enhanced capacity of passive sensitization, the ratio between RAST and passive sensitization capacity increased significantly in *Cladosporium*-treated patients, implying a less than expected sensitization capacity of immunotherapy-induced specific IgE. The lack of active binding of IgE to basophils might be explained by a reduced Fc-affinity of immunotherapy-induced IgE in contrast to the *Cladosporium*-seasonally induced IgE. Regarding the decrease in histamine release in *Cladosporium*-treated patients in spite of an increased amount of cell-bound specific IgE, immunotherapy may initiate a decrease in mediator releasability which is not caused by a reduction in the number of Fc-receptors but rather some yet unknown subcellular mechanisms regulating the histamine release. The described changes in IgE-mediated parameters do not seem to be caused by interference with either specific IgG₁ or IgG₄. Changes in histamine release in the *Cladosporium* season were the only IgE-mediated parameter significantly related to the graded clinical efficacy of immunotherapy.

Key words: *Cladosporium* allergy; clinical efficacy; histamine release; immunotherapy; passive sensitization; specific IgE.

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The therapeutic mechanisms of allergen-specific immunotherapy are still a subject of debate. Several immunological mechanisms have been proposed as candidates, including blocking antibodies, reduced production of IgE, and reduced release of allergic mediators from mast cells (6). The pathogenic role of IgE in allergic sensitization and release of mediators is well established (8, 13, 14). The interrelationship between IgE

and clinical efficacy during immunotherapy is controversial, especially the role of circulating IgE. Most immunotherapy studies show an early intense burst of IgE and then declining values that do not always fall below pre-treatment values (1, 16, 23, 30).

In an uncontrolled pilot study (19) we have previously offered the explanation that immunotherapy-induced IgE had a low cell-sen-

sensitizing capacity (low Fc-receptor affinity) and thereby has no pathogenic role. The present study deals with the relation between histamine releasing capacity of the circulating basophil leucocytes, serum and basophil cell-bound specific IgE, and the sensitizing capacity of serum IgE. Immunotherapy-induced changes are compared with natural changes caused by seasonal allergen exposure in 11 *Cladosporium*-treated versus 11 placebo-treated asthmatics.

MATERIAL AND METHODS

Patients

Twenty-two adult asthmatics participating in a placebo-controlled double-blind immunotherapy study with *Cladosporium* were investigated. The patients were randomized according to SPT and BPT sensitivity and showed no significant difference with respect to RAST value, age, sex, duration of mould allergy, associated allergies, and severity of asthma. Detailed information concerning diagnostic procedures, immunotherapy, and evaluation of clinical efficacy have previously been reported (21, 22). A significant ($P = 0.01$) difference was found in terms of improved and unchanged clinical condition (81% in the *Cladosporium* group and 27% in the placebo-treated) versus deterioration in disease severity (19% and 73% respectively).

Allergen extracts

The same batch of a lyophilised, partially purified, biologically standardized extract of *Cladosporium herbarum* (Pharmalgen®, Pharmacia AB, Uppsala, Sweden) as used for immunotherapy was employed in all the *in vitro* studies. Stability and composition were controlled by quantitative immunoelectrophoresis.

Blood samples

Venous blood was obtained for histamine release and estimation of serum as well as basophil-bound IgE before treatment, at maintenance dose (preseasonally), after the *Clado-*

sporium season, and after 1 year of immunotherapy.

Histamine release was performed using isolated mononuclear leucocytes as previously described (21).

Mononuclear cells from 50 ml blood were isolated by Ficoll-Hypaque density gradient technique and IgE dissociated from basophils by adjusting pH in the leucocyte-suspension from 7.2–4.0 (33). The cell-free supernatant was dialysed and lyophilised for subsequent IgE-determination

Estimation of specific IgE

Specific IgE in serum was measured by paper RAST technique as previously described (21, 23).

Basophil surface IgE was quantitated by an ultrasensitive Maxisorp RAST developed at our laboratory (28). 300 μ l allergen extract diluted to 5,000 BU/ml in phosphate buffer were added to test tubes (Maxisorp polystyrene tubes) and incubated at room temperature overnight. The tubes were washed four times in 1% Tween phosphate buffer. The lyophilised IgE-rich fraction eluted from basophils was reconstituted in 300 ml phosphate buffer and incubated overnight in allergen-coated test tubes. After washing four times, 300 μ l ^{125} I-anti-IgE (Phadebas® isotop, Pharmacia AB, Uppsala, Sweden) diluted to approximately 10,000 cpm were added and incubated overnight before counting. All samples were tested in one set up.

Specific IgE in samples was compared with a reference standard curve made by serial dilution of basophil surface IgE from one of the patients. The standard curve of eluted IgE was parallel with a serum standard curve and showed a linear response (log-log) down to 0.1 SU/ml ($r = 0.994$). Unpublished data from our laboratory have shown one RAST-SU to be equivalent to 3.8 ng specific IgE. The reproducibility of double determination showed a coefficient of variation of 3.5. The specificity was controlled by no uptake of non-*Cladosporium* IgE and inhibition by preincubation with *Cladosporium* allergen only. Cell-bound specific IgE was expressed as SU per 10^6 basophils (33).

Passive sensitization of basophils

Passive sensitization was carried out using basophils isolated from a single non-allergic donor who in preliminary testing had shown a high capacity for sensitization. Autologous IgE was removed by acid treatment as previously described (34). Undiluted serum drawn from patients participating in the immunotherapy study was incubated with "stripped" cells for 2 h at 37°C. After washing, basophils were challenged with *Cladosporium* in 10-fold dilutions from 0.1 to 100 BU/ml and four 10-fold dilutions of anti-IgE (Behringwerke, W. Germany). To compare the sensitization capacity of serum from various patients, the histamine release was quantitated as the area under the curve (AUC) tested within four 10-fold dilutions of both *Cladosporium* and anti-IgE. The area was calculated by planimetry (20).

Statistics

Within-group comparisons were performed by Wilcoxon's test and between-groups by Mann-Whitney's U-test. Correlations were calculated by Spearman's test.

RESULTS

Specific IgE in serum

As previously described (23), no significant difference was found between pretreatment serum-specific IgE (RAST) in the *Cladosporium* and the placebo group. In the former group median RAST increased to more than double the initial value (from 27 to 62 SU/ml) at cluster IV, i.e. after 6 weeks of treatment, and declined thereafter steadily to 1.5 of the pretreatment value (40 SU/ml) after 1 year of immunotherapy. The placebo group showed no significant changes.

Basophil cell-bound specific IgE

No significant difference in cell-bound IgE was found between the treatment and the pla-

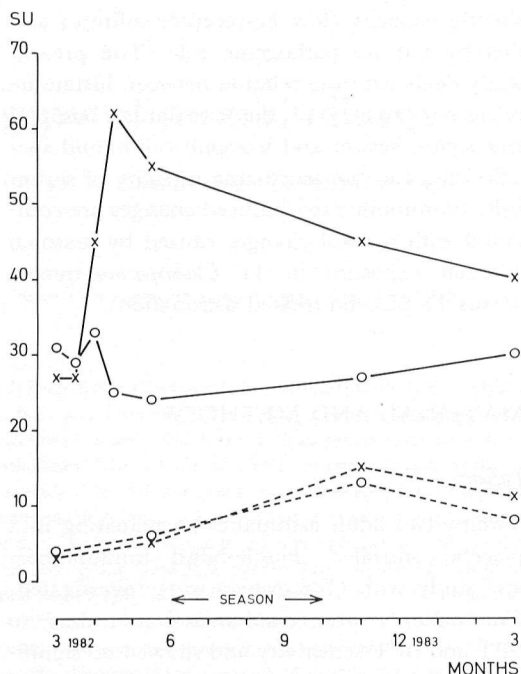


Fig. 1. Median values of serum-specific IgE (RAST SU/ml) (fully drawn line) and basophil cell-bound specific IgE (SU/10⁶ basophils) (stippled line) during the first year of immunotherapy. x = *Cladosporium*-treated patients and o = placebo group.

cebo group during the first year of treatment. From pretreatment to pre-season the amount of specific IgE did not change. During the season, the median value increased 3-fold in the *Cladosporium* group and a little less in the placebo group ($P < 0.01$). A slight decline was observed after the season.

Discordance of serum and cell-bound IgE

In the *Cladosporium* group a clear discordance was observed between the intense rise in serum-specific IgE during the dose-increase phase of immunotherapy and unchanged amount of basophil cell-bound specific IgE (Fig. 1). Likewise, a discordance was found between the seasonal increase in cell-bound IgE and the decrease in circulating IgE. As regards the placebo group, basophil cell-bound

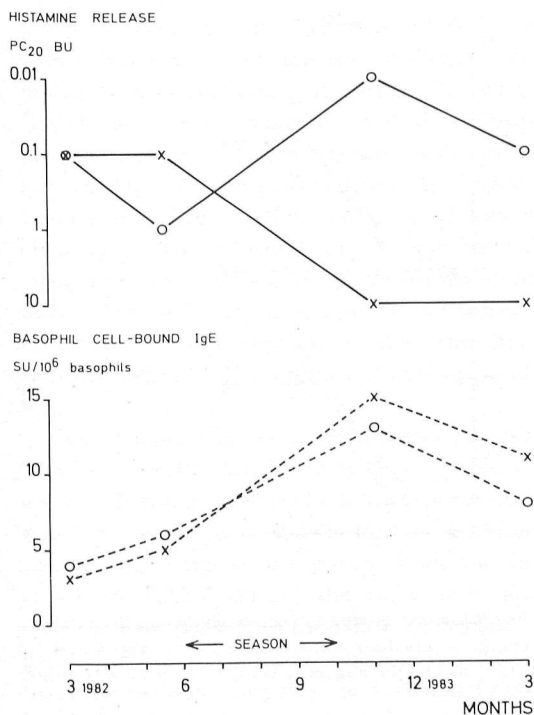


Fig. 2. Histamine release cell sensitivity (PC₂₀ BU) and basophil cell-bound specific IgE (SU/10⁶ basophils) during the first year of immunotherapy. x = *Cladosporium*-treated patients and o = placebo group.

IgE to a certain degree paralleled changes in serum values.

Histamine release from basophil leucocytes

Histamine release (PC₂₀) in the *Cladosporium* group did not change from pretreatment to pre-season. During the season, the median sensitivity decreased two log steps (From 0.1 to 10 BU) ($P < 0.02$), a value that was stable in the remaining observation period (Fig. 2). The placebo group, on the contrary, showed a decreased sensitivity of one log step in the preseason sample. During the season, the sensitivity increased two log steps (from 1 to 0.01 BU) ($P < 0.02$). After the season PC₂₀ returned to the pretreatment value. Only samples taken after 1 year of treatment showed a marginally significant ($P = 0.05$) difference between the two groups.

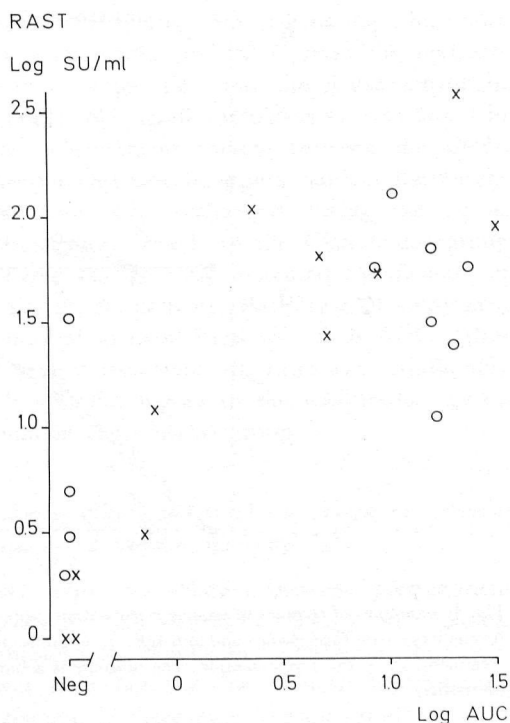


Fig. 3. Relation between pretreatment serum-specific IgE (RAST log SU/ml) and the passive sensitization capacity of pretreatment sera. Sensitization capacity is expressed as the area under the curve (AUC) of *Cladosporium*-induced histamine release. x = *Cladosporium*-treated patients and o = placebo group.

Discordance of basophil cell-bound IgE and histamine release

In the *Cladosporium* group no discordance was seen between basophil-bound IgE and histamine release during dose-increase immunotherapy (Fig. 2). In the *Cladosporium* season, however, the median sensitivity estimated by histamine release was reduced two log steps in spite of a three-doubling of basophil cell-bound specific IgE. In the placebo group partly concordant changes were observed.

Sensitization capacity of serum IgE

Estimated by anti-IgE challenge, passive sensitization and histamine release, three patients

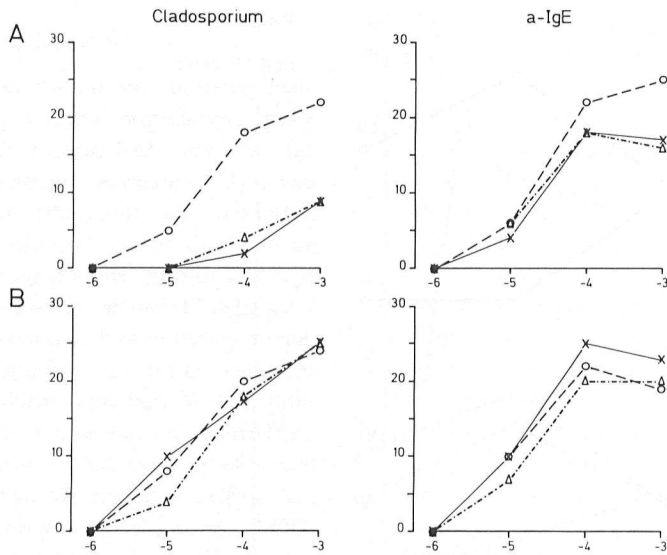


Fig. 4. Examples of changes in passive sensitization capacity in the *Cladosporium* group (A) and placebo group (B) estimated by challenge with *Cladosporium* and anti-IgE. x = pretreatment sera, O = sera drawn before cluster IV, i.e. after 6 weeks of treatment, Δ = the 1-year-sample. The ordinate is arbitrary AUC units and the abscissa gives the allergen and anti-IgE-dilutions.

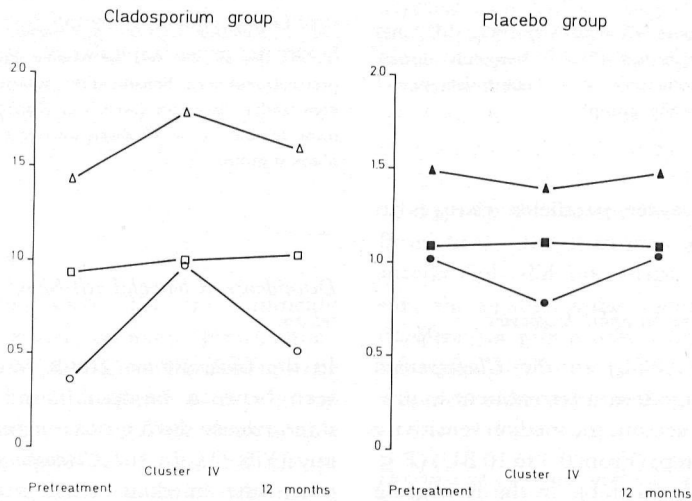


Fig. 5. Median values of serum-specific IgE (RAST SU/ml) (Δ). Sensitization capacity of serum estimated by anti-IgE challenge (□) and *Cladosporium* challenge (O) in samples taken before treatment, at cluster IV, and after 1 year of treatment. The ordinate is arbitrary units (log SU/ml and log AUC).

(27%) in the *Cladosporium* group and four (36%) in the placebo group could not be passively sensitized. This phenomenon was caused in all but one (placebo-treated) patient by very

low RAST-values. As shown in Fig. 3 there is a certain degree of saturation kinetic but with a wide variation in the sensitizing capacity of serum with the same RAST-value.

Fig. 4 shows a typical response of AUC to both *Cladosporium* and anti-IgE challenge estimated before treatment, at cluster IV (when RAST-values were maximal), and after 1 year of treatment in a patient treated with *Cladosporium* (Fig. 4A) and placebo (Fig. 4B). In the *Cladosporium* group, the AUC (*Cladosporium* challenge) was increased in the preseasonal sample and then returned to pretreatment levels after 1 year of immunotherapy. In the placebo group no changes were observed in the three samples, either by *Cladosporium* or anti-IgE challenge.

The relation between serum-specific IgE (RAST) and the sensitization capacity of circulating IgE estimated by both *Cladosporium* and anti-IgE challenge is shown in Fig. 5. Concerning the *Cladosporium* group, both the increase in RAST during the immunotherapy dose-increase phase and the following decline is paralleled by the *Cladosporium*-induced histamine release which opposes the unchanged anti-IgE-induced release. In the placebo group, the same tendency was observed with the reservation that the preseason sample (cluster IV) showed the lowest RAST-value and *Cladosporium*-induced histamine release.

However, some discrepancy was observed in the *Cladosporium* group between changes in serum-IgE and AUC during the dose-increase phase. The AUC was unchanged in 4/6 patients with RAST-values increasing > 100 SU/ml. Two patients with increased RAST-values of < 100 SU/ml both showed an AUC-increase. The interaction of IgG₄ either acting as a blocking or a sensitizing (short-term sensitizing) antibody on this apparent discrepancy was evaluated by comparing both the absolute levels and the changes in IgG₄ in the periods in question. A possible sensitizing role of IgG₄ was observed in two patients, although the increase in AUC might simply be explained by an increase in RAST. A possible blocking capacity of IgG₄ was observed only in one patient in whom the unchanged AUC, in spite of a marked increase in RAST, might be caused by an increase in IgG₄.

The apparently low sensitizing capacity of the immunotherapy-induced specific IgE was

further evaluated by calculating the ratio between RAST and AUC from the pretreatment, cluster IV, and the 1-year-treatment sample. No significant difference was found in the pretreatment sample between the *Cladosporium* and placebo group, and in the latter the ratio was unchanged during the 1-year observation period. In the *Cladosporium* group (Table 1), the ratio increased significantly in half of the patients, indicating a more pronounced increase in RAST than AUC. After 1 year of treatment, the ratio was considerably ($P = 0.05$) higher in the *Cladosporium* group than in the placebo group.

Clinical efficacy and relation to changes in histamine release and basophil cell-bound IgE

We found no relation between pretreatment basophil cell sensitivity (PC₂₀) and clinical efficacy. A significant correlation, however, was seen between the clinical outcome and changes in histamine release from pretreatment to postseason (Rho 0.74, $P < 0.05$) and partly from preseason to postseason (Rho 0.68, $P = 0.05$) (Fig. 6). Neither absolute values nor changes in basophil cell bound IgE were related to the clinical efficacy of immunotherapy.

DISCUSSION

Good agreement has been reported between serum-specific IgE, basophil cell-bound IgE and histamine release cell-sensitivity (allergen concentrations eliciting release of a fixed per-

Table 1

Ratio between serum specific IgE (RAST) and cell-sensitizing capacity of serum IgE (AUC) estimated by *Cladosporium* challenge

	Pretreatment	Cluster IV	1 year treatment
<i>Cladosporium</i> group	6.5	14.5	16.3
Placebo group	2.4	3.5	2.3
Median values			

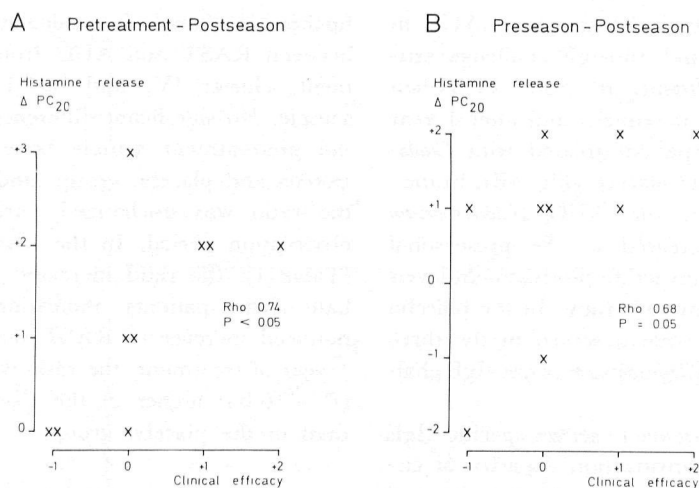


Fig. 6. Relation between the graded clinical efficacy of *Cladosporium* immunotherapy and changes in histamine release cell sensitivity (PC_{20} log BU) pretreatment to postseason (A) and preseason to postseason (B).

centage of total histamine content) in non-immunotherapy-treated allergics (12, 25, 33). Some individual variations in histamine release in spite of a constant number of cell-bound IgE molecules have been reported (3, 18, 26, 29). This phenomenon has led to the introduction of the term "basophil releasability" (3, 18), a genetical characteristic of the basophils to release mediators probably determined by subcellular mechanisms (18) of which one may be the calcium gating factor (5).

Immunotherapy might induce a reduction in basophil histamine release (16) and in a few patients basophils might become non-reactive (30). In an uncontrolled pilot study, a fundamental discordance between serum-specific IgE, basophil cell-bound IgE and histamine release has been revealed (19). The present study is a further extension of these preliminary results including a control group.

Circulating and basophil-bound IgE

Serum-specific IgE in *Cladosporium*-treated patients showed an early (within 6 weeks of immunotherapy) response and then decreasing values both during the season and postseason-

ally. This is in contrast to the number of basophil-bound specific IgE molecules that increased significantly in the *Cladosporium* season. These inverse changes appear to be explained by the fact that IgE induced early in the course of immunotherapy does not bind to basophils. This might be caused by at least two mechanisms: firstly, Fc-receptors are already preoccupied by IgE and secondly, the affinity to Fc-receptors of immunotherapy-induced IgE is low. As regards the former possibility, several studies have shown the presence of free Fc-receptors (12, 25, 33, 38). Furthermore, the presented data showing a three-doubling in basophil-bound IgE during the season exclude the possibility of preoccupied Fc-receptors. The latter hypothesis, however, is favoured by several data. From a clinical point of view, a manifold increase of the number of basophil-bound specific IgE molecules might induce a state of extreme hyperreactivity that would make further dose increase impossible due to systemic side effects. As shown in a previously published paper (22), the number of systemic side effects does not increase concordantly with the RAST-increase. The affinity of IgE to the Fc-receptor varies between 10^9 to $10^{10}/M$ (association constant) in untreated patients but with

a wide individual variation (12, 25). This variation might reflect a heterogeneity in the Fc-receptor as well as the Fc-portion of the IgE molecule (4, 25). No affinity studies during immunotherapy have favoured either of the possibilities. The present passive sensitization experiments indicate a causative functional heterogeneity in IgE molecule (4). Using recombinant DNA technology in subcloning the 2nd, 3rd and 4th domains of the heavy chain of human IgE, Geha et al. (9) found distinct binding sites for mast cells and other cell types that might be taken into account as heterogeneity on the molecular level. Likewise, a functional heterogeneity in the IgE-producing B cell pool has been reported (36). Our theory is that immunotherapy – in contrast to the natural seasonal exposure to allergens – favours a production of IgE with a low affinity to the Fc-receptor. The seasonal increase in basophil-bound IgE in both treatment groups is reflected in serum values in the placebo group. These findings are in agreement with previously published data (19) and might be caused by an enhanced *in vitro* synthesis from human peripheral mononuclear cells during the *Cladosporium* season as observed by Turner et al. (37). Grammer et al. (10), however, did not find seasonal changes in cell-bound IgE.

The passive sensitization experiments were performed to further evaluate the affinity of IgE to basophils during immunotherapy, a phenomenon hitherto uninvestigated. The use of cells from only one donor excludes variations in the Fc-receptor number and function (29). We found a passive sensitization capacity estimated by anti-IgE challenge in all sera, and a specific IgE sensitization in 2/3 of the patients. The lack of specific sensitization was for practical purposes caused by low levels of serum-specific IgE (29). As shown in several passive sensitization studies, the sensitization capacity of serum with equal levels of specific IgE varies considerably (4, 26, 29, 38). This phenomenon does not reflect retained autologous IgE as the cells have been acid-treated (17, 29). The ratio of specific/total IgE is important as irrelevant IgE suppresses the passive sensitization (38). Individual variation in the distribution of IgE to various

epitopes in the *Cladosporium* extract might result in variations in passive sensitization (26). Our data show that the majority of patients with a marked (> 100 SU/ml) increase in serum-specific IgE does not have an increased sensitization capacity, supporting the theory of a low Fc-affinity of immunotherapy-induced IgE. Our data do not exclude an inhibitor of sensitization or basophil cell function as the causative mechanism as suggested by Pruzansky et al. (29).

Histamine release and specific IgE

In the placebo group we found concordant variation of *Cladosporium*-induced histamine release and basophil-bound specific IgE. In the season both parameters increased due to allergen exposure and values then decreased in the postseasonal period (Fig. 2). These fluctuations have been reported earlier (32) but not consistently (10). In the *Cladosporium*-treated patients, cell sensitivity was reduced after a lag phase, i.e. more allergen was required for a 20% histamine release in spite of an increasing amount of basophil cell-bound specific IgE (19). The immunotherapy-induced reduced histamine release is confirmed by other studies (16, 30). These changes must include the concept of basophil releasability (18). According to the presented results, this mechanism does not seem a function of decrease in IgE-receptors (5, 12, 16, 33) but rather is caused by some subcellular histamine release controlling mechanisms (18, 19). Factors, probably immune-specific lymphokines, known to modulate the histamine release have been described both in terms of enhancing (2, 11) and reducing the histamine release (27). The inhibitory activity has only been described in plasma from non-atopic persons (27) but similar immune regulatory mechanisms could be induced by immunotherapy.

Interference of IgG

No or only a marginal role of specific IgG-subclasses was revealed in the present study. Two potential mechanisms of IgG might regulate the allergic response. The allergen could be

inhibited by IgG before obtaining access to the IgE-bearing mast cells. This would require high-affinity IgG-antibodies, and based on *in vitro* experiments it does not seem an important mechanism (31). Based on results of the clinical efficacy of *Cladosporium* immunotherapy, a high specific IgG antibody level is associated with a deleterious effect of treatment (24). The other mechanism is a sensitizing capacity of IgG₄ as claimed by a number of investigators (15, 35, 38) although questioned by others (39). As we have shown a decrease of histamine release contrasting the increase of serum IgG₄, sensitization of basophils by IgG₄ does not seem important.

Mediator release and relation to the clinical efficacy of immunotherapy

The presented data show a direct, although not impressive, relation between changes in histamine release and clinical efficacy. This relation does not *per se* reveal fundamental immunological mechanisms as the asthma disease is primarily caused by bronchoalveolar mast cells and not circulating basophils. Some support for parallel changes in basophils and lung mast cells has, however, been reported by Flint et al. (7). Furthermore, basophils seem to infiltrate the area of active inflammation (18). The clinical efficacy of specific immunotherapy might (in the initial phase) be caused by an *in vivo* desensitization (relatively) due to the continuous exposure of allergens under suboptimal conditions that do not involve removal of surface IgE or Fc-receptors by shedding or endocytosis (18). The study of Sadan et al. (30) clearly shows a relation between histamine release and hay fever symptoms as the patients with the greatest decrease in histamine release essentially became symptom free.

Conclusion

The present data reveal some regulatory mechanisms regarding circulating IgE, basophil-bound IgE, and histamine release caused by seasonal allergen exposure as well as immunotherapy. With the limitation that only one allergen system and few patients are investigated, these mechanisms might be of importance

in understanding the fundamental immunological mechanisms involved in the clinical efficacy of immunotherapy. We have shown that the immunotherapy-induced burst of circulating specific IgE is not reflected in the number of specific IgE-molecules bound to basophils, probably due to a low Fc-receptor affinity of circulating IgE. Furthermore, the decline of histamine release during immunotherapy, that correlates with the graded clinical response, is not a consequence of a reduction of basophil cell-bound specific IgE but rather represents a decreased releasability of basophils, the nature of which is at the moment unknown.

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REFERENCES

1. Ahlstedt, S. & Eriksson, N. E.: Immunotherapy in atopic allergy - antibody titres and avidities during hyposensitization with birch and timothy pollen allergens. *Int. Arch. Allergy. Appl. Immunol.* 55, 400-411, 1977.
2. Bamzai, A. K. & Kretschmer, R. R.: Enhancement of antigen-induced leukocyte histamine release by a mononuclear cell-derived factor. *J. Allergy Clin. Immunol.* 62, 137-142, 1978.
3. Conroy, M. C., Adkinson Jr. N. F. & Lichtenstein, L. M.: Measurement of IgE on human basophils: Relation to serum IgE and anti-IgE-induced histamine release. *J. Immunol.* 118, 1317-1321, 1977.
4. Conroy, M. C., Adkinson Jr., N. F. & Lichtenstein, L. M.: Passive sensitization of human basophils: Evidence for heterogeneity in the IgE molecule. *Int. Arch. Allergy Appl. Immunol.* 60, 106-109, 1979.
5. Dembo, M. & Goldstein, B.: A model of cell activation and desensitized by surface immunoglobulin: the case of histamine release from human basophils. *Cell* 22, 59-67, 1980.
6. Filipp, G.: Specific desensitization and its mechanisms. *Allergol. Immunopathol.* 10, 305-318, 1982.
7. Flint, K. C., Leung, K. B. P., Hudspeth, B. N., Brostoff, J., Pearce, F. L., Johnson, N. M.: Bronchoalveolar mast cells in extrinsic asthma: a mechanism for the initiation of antigen specific bronchoconstriction. *Br. Med. J.* 291, 923-926, 1985.
8. Geha, R. S.: Human IgE. *J. Allergy Clin. Immunol.* 74, 109-120, 1984.

9. Geha, R. S. & Leung, D. Y. M.: Regulation of the human allergic response. *Int. Arch. Allergy Appl. Immunol.* **82**, 389-391, 1987.
10. Grammer, L., Levitz, D., Roberts, M., Pruzansky, J. J. & Zeiss, C. R.: Seasonal variation of IgE antibody specific for ragweed antigen E (IgE-a-AgE) from the basophil surface in patients with ragweed pollinosis. *Int. Arch. Allergy Appl. Immunol.* **66**, 179-188, 1981.
11. Ida, S., Hooks, J. J., Siraganian, R. P. & Notkins, A. L.: Enhancement of IgE-mediated histamine release from human basophils by immune-specific lymphokines. *Clin. Exp. Immunol.* **41**, 380-387, 1980.
12. Ishizaka, T., Soto, C. S. & Ishizaka, K.: Mechanisms of passive sensitization. III. Number of IgE molecules and their receptor sites on human basophil granulocytes. *J. Immunol.* **111**, 500-511, 1973.
13. Ishizaka, T.: IgE and mechanisms of IgE-mediated hypersensitivity. *Ann. Allergy* **48**, 313-319, 1982.
14. Johansson, S. G. O. & Bennich, H. H.: The clinical impact of the discovery of IgE. *Ann. Allergy* **48**, 325-330, 1982.
15. Kimura, I., Tanizaki, U., Sato, S. & Takahashi, K.: Differences in response to anti-IgE and to anti-IgG in basophils from patients with bronchial asthma. *Clin. Allergy* **11**, 31-36, 1981.
16. Kimura, I., Tanazaki, Y., Goda, Y., Komagoe, H. & Kitani, H.: Decrease in reactivity of basophils by immunotherapy with housedust extract. *Clin. Allergy* **15**, 1-17, 1985.
17. Levy, D. A. & Osler, A. G.: Studies on the mechanisms of hypersensitivity phenomena. XIV. Passive sensitization in vitro of human leukocytes to ragweed pollen antigen. *J. Immunol.* **97**, 203-212, 1966.
18. Lichtenstein, L. M. & MacGlashan, D. W.: The concept of basophil releasability. *J. Allergy Clin. Immunol.* **77**, 291-294, 1986.
19. Mallng, H.-J., Stahl Skov, P., Permin, H., Norn, S. & Weeke, B.: Basophil histamine release and humoral changes during immunotherapy. *Allergy* **37**, 187-190, 1982.
20. Mallng, H.-J.: Diagnosis and immunotherapy of mould allergy. II. Reproducibility and relationship between skin sensitivity estimated by end-point titration and histamine equivalent reaction using skin prick test and intradermal test. *Allergy* **40**, 354-362, 1985.
21. Mallng, H.-J., Dreborg, S. & Weeke, B.: Diagnosis and immunotherapy of mould allergy. III. Diagnosis of Cladosporium allergy by means of symptom score, bronchial provocation test, skin prick test, RAST, CRIE and histamine release. *Allergy* **41**, 57-67, 1986.
22. Mallng, H.-J., Dreborg, S. & Weeke, B.: Diagnosis and immunotherapy of mould allergy. V. Clinical efficacy and side effects of immunotherapy with Cladosporium herbarum. *Allergy* **41**, 507-519, 1986.
23. Mallng, H.-J., Dreborg, S. & Weeke, B.: Diagnosis and immunotherapy of mould allergy. VI. IgE-mediated parameters during a one-year placebo-controlled study of immunotherapy with Cladosporium. *Allergy* **42**, 305-314, 1987.
24. Mallng, H.-J. & Djurup, R.: Diagnosis and immunotherapy of mould allergy. VII. IgG subclass response and relation to the clinical efficacy of immunotherapy. *Allergy* **43**, 60-70, 1988.
25. Malveaux, F. J., Conroy, M. C., Adkinson Jr., N. F. & Lichtenstein, L. M.: IgE receptors on human basophils. Relationship to serum IgE concentration. *J. Clin. Invest.* **62**, 176-181, 1978.
26. Mao, J. Le, Weyer, A., Dandeu, J. P., Rabillon, J. & David, B.: Relationship between specific circulating IgE, basophil cell-bound IgE and histamine release induced by purified allergens of *Dermatophagoides farinae*. *Int. Arch. Allergy Appl. Immunol.* **76**, 289-295, 1985.
27. Miadonna, A., Tedeschi, A. & Zanussi, C.: Plasma of normal, but not atopic persons reducing basophil degranulation induced by anti-IgE. *Clin. Allergy* **14**, 29-35, 1984.
28. Poulsen, L. K., Pedersen, M. F., Mallng, H.-J., Søndergaard, I. & Weeke, B.: Maxisorp RAST - A sensitive method for detection of absolute quantities of antigen-specific human IgE. *J. Immunol. Methods*. (in press).
29. Pruzansky, J. J., Grammer, L. C., Patterson, R. & Roberts, M.: Dissociation of IgE from receptors on human basophils. *J. Immunol.* **131**, 1949-1953, 1983.
30. Sadan, N. S., Rhyne, M. B., Mellits, E. D. et al.: Immunotherapy of pollinosis in children. Investigation of the immunologic basis of clinical improvement. *N. Engl. J. Med.* **280**, 623-627, 1969.
31. Schumacher, M. J. & Jeffery, S. E.: Effect of quantity and quality of IgG antibodies on blocking of allergenic histamine release in vitro. *Int. Arch. Allergy Appl. Immunol.* **58**, 38-43, 1979.
32. Stahl Skov, P., Norn, S. & Weeke, B.: Basophil histamine release in patients with hay fever. Results compared with specific IgE and total IgE during immunotherapy. *Clin. Exp. Immunol.* **27**, 432-439, 1977.
33. Stahl Skov, P., Permin, H. & Mallng, H.-J.: Quantitative and qualitative estimations of IgE bound to basophil leukocytes from hay fever patients. *Scand. J. Immunol.* **6**, 1021-1028, 1977.
34. Stahl Skov, P., Jensen, C., Nolte, H., Mosbech, H. & Norn, S.: Regulatory role of cell-bound IgE on histamine release. In Schou, J. S., Geisler, A. & Norn, S. (eds.): *Drug receptors and dynamic processes in cells*. Alfred Benzon Symposium 22. Munksgaard, Copenhagen, 1986.
35. Stanworth, D. R.: The molecular pathology of IgG4. *Monogr. Allergy* **19**, 227-240, 1986.
36. Turner, K. J., Holt, B. J., Cameron, K. J. & Holt, P. G.: Functional heterogeneity with the human peripheral blood B cell pool engaged in IgE synthesis. *Int. Arch. Allergy Appl. Immunol.* **82**, 394-397, 1987.
37. Turner, K. J., Siemensma, N. P., Krska, K., Cameron, K. J.: Seasonal variations in in vitro synthesis of rye pollen-specific IgE by human peripheral mononuclear cells. *Int. Arch. Allergy Appl. Immunol.* **82**, 398-401, 1987.
38. Toorenenbergen van, A. W. & Aalberse, R. C.: Aller-

gen-specific IgE and IgG4 antibody levels in sera and the capacity of these sera to sensitize basophil leucocytes in vitro. Clin. Allergy 12, 451-458, 1982.

39. Zhou, T., Conroy, M. C., Spengler, H., de Weck, A. L: Failure of covalently cross-linked human IgG myeloma subclass protein to release histamine from human leukocytes. Int. Arch. Allergy Appl. Immunol. 74, 108-112, 1984.

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