

Basophil Histamine Release and Humoral Changes during Immunotherapy

Dissociation between Basophil-Bound Specific IgE, Serum Value, and Cell Sensitivity

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An *in vitro* pilot study was performed to determine whether basophil cell-bound specific IgE was correlated to serum-specific IgE and to basophil cell sensitivity during immunotherapy with a well-documented treatment. The findings showed a clear dissociation between these three parameters, which is in contrast to the situation before hyposensitization and to our previous study in a comparable group of non-hyposensitized patients, where highly significant correlations were observed. Further investigations are in progress to clarify whether the dissociation is involved in the clinical improvement.

Key words: basophil cell-bound specific IgE; cell sensitivity; immunotherapy; RAST; timothy grass pollen.

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Immunotherapy with pollen allergen extracts is a well-documented treatment of patients with allergic rhinitis and asthma (4, 13). Specific immunotherapy initiates changes in several *in vitro* parameters including an initial rise of serum-specific IgE followed by a decrease (2, 3, 6, 9, 11). Changes of the same nature are seen in basophil cell sensitivity measured by histamine release technique (2, 6, 11).

In a previous study we have investigated the relationship between basophil cell-bound specific IgE, serum values (RAST), and cell sensitivity. In non-hyposensitized grass pollen hay fever patients a highly significant linear correlation was found between cell-bound IgE and both RAST and cell sensitivity (12). These results suggested that a steady state between serum-specific and basophil-bound IgE was

reached and that the basophil cell sensitivity was determined by the number of cell-bound specific IgE molecules (12).

In order to elucidate whether basophil cell-bound IgE was correlated to RAST and cell sensitivity during immunotherapy, an *in vitro* pilot study was performed. Hyposensitization was performed by the well-documented treatment of Østerballe (13). Surprisingly, it was found that basophil-bound IgE was not correlated to the other two parameters – a finding which suggests that the said hypothesis concerning IgE and the basophil leukocyte in non-hyposensitized patients (12) might not be valid for hyposensitized individuals. Whether the observed dissociation is involved in the clinical improvement is not clarified, and further investigations are in progress.

MATERIAL AND METHODS

Patients

Seven timothy grass pollen allergic rhinitis patients with a well-established specific diagnosis were investigated. No patients had received immunotherapy earlier, and during the investigation no systemical corticosteroids were administered. Immunotherapy was performed routinely with a partly purified alum-precipitated timothy grass pollen extract (Allergologisk Laboratorium A/S, Copenhagen, Denmark). Hyposensitization was initiated from December 1977 to March 1978. Injections were given weekly until maintenance dose and continued every second month all the year round. All patients received the maximally tolerated dose.

Estimation of specific IgE

Specific IgE in serum was measured by RAST technique according to Ceska et al. (1). Paper discs were used as a solid phase, and the results based upon the counts are expressed in sorbent units (SU).

Surface IgE from basophils was tested in essentially the same way. IgE was removed from the cell surface by adjusting pH in the leukocyte suspension from 7.2 to 4.0. After centrifugation, the IgE-containing supernatant was concentrated by a Minicon-B concentrator (Amicon), and specific IgE was assayed as described by Stahl Skov et al. (12).

Histamine release

Mononuclear cells were isolated by the Ficoll-Hypaque density gradient technique and the cell suspension was incubated with various concentrations of timothy grass pollen extracts (10). The release of histamine was expressed as a percentage of the total histamine content of the sample. Correction was made for spontaneous histamine release. The cell sensitivity was expressed as the pollen concentration giving a 20% histamine release (HR20) (2, 11).

Statistics

Wilcoxon signed rank sum test for matched pairs was used to compare the examined parameters before and during treatment.

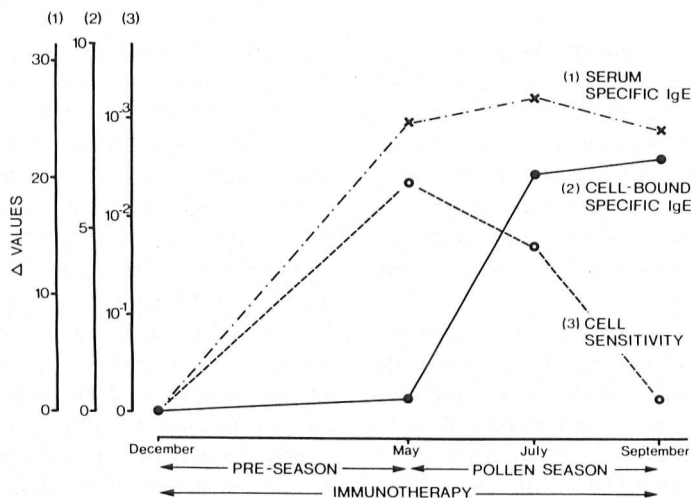


Fig. 1. Dissociation between serum-specific IgE (SU/ml), basophil-bound specific IgE (SU/10⁶ cells), and cell sensitivity (HR20). Mean Δ values of the three parameters are indicated at four dates, before the start of immunotherapy, immediately before the grass pollen season, in the middle and just after the season.

RESULTS AND DISCUSSION

From Fig. 1 it appears that serum-specific IgE increased in all the patients from the start of the immunotherapy until the middle of May ($P = 0.01$). During the grass pollen season (end of May to September) no significant change was obtained in IgE, although a slight increase was followed by a decrease.

The cell sensitivity tended to increase until the grass pollen season ($P = 0.05$). During the grass pollen season five of the patients showed a decline in cell sensitivity, while two showed no change.

The observed changes in serum-specific IgE and cell sensitivity are in accordance with those found in other investigations (2, 6, 11), which also showed that in untreated hay fever patients both RAST and cell sensitivity were often increased during the pollen season. The reduced cell sensitivity by immunotherapy in the pollen season was therefore suggested to be caused by a reduced IgE production causing smaller amounts of specific IgE to be bound to the target cell (11). However, as also indicated in our previous paper (11), the hypothesis did not rule out a co-operative involvement of other cellular mechanisms.

The number of specific IgE molecules bound to the cell surface of the basophil leukocyte has not earlier been followed during immunotherapy. The present investigation showed that cell-bound IgE did not change during the pre-seasonal period (Fig. 1).

During the first half of the pollen season a significant increase in basophil-bound IgE was observed in all seven patients ($P = 0.01$). Thereafter the picture varied during the last part of the season. Two patients showed no change in cell-bound IgE, while three showed a decrease and two an increase of specific IgE.

The findings (Fig. 1) show a clear dissociation between cell sensitivity and cell-bound IgE during the pre-seasonal period ($P = 0.05$) and the pollen season ($P < 0.05$), and a similar dissociation was also observed between serum IgE and basophil-bound IgE in the pre-seasonal period ($P < 0.01$), and in the first part of the grass pollen season ($P = 0.01$).

The dissociation between cell sensitivity and cell-bound IgE was unexpected. It suggests that factors other than the density of IgE molecules on the cell membrane might modulate the sensitivity of the target cell to antigen. Such factors may play a major role in hyposensitized patients. We do not know these factors, but the observed dissociations suggest a change in the subcellular biochemical events leading to histamine release – events which have already been brought into focus in connection with *in vivo* and *in vitro* hyposensitization (5, 7, 8). Also a change in the affinity of specific IgE to IgE receptors or a change in the receptor density might be involved in the dissociation between the three parameters.

Our preliminary study of the dissociation in hyposensitized patients does not include a control group. However, in a previous study of a comparable group of non-immunotherapy treated patients we found no dissociation, but a highly significant correlation between these parameters (12). Such a correlation was also found in the patients of the present study before start of the hyposensitization ($r = 0.81$, $P = 0.03$), but during the hyposensitization this correlation was converted into a clear dissociation. It therefore seems reasonable to suggest that the dissociation might be caused by the immunotherapy. Further investigations are in progress to clarify whether the observed dissociation is involved in the clinical improvement.

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REFERENCES

1. Ceska, M., Eriksson, R. & Varga, J. M.: Radioimmunosorbent assay of allergens. *J. Allergy Clin. Immunol.* **49**, 1–9, 1972.
2. Irons, J. S., Pruzansky, J. J., Patterson, R. & Zeiss, C. R.: Studies of perennial ragweed immunotherapy. Associated changes in cellular responsiveness, total serum antigen-

- binding capacity, and specific IgE antibody concentrations. *J. Allergy Clin. Immunol.* 59, 190–199, 1977.
3. Johansson, S. G. O., Miller, A. C. M. L., Overell, B. G. & Wheeler, A. W.: Changes in serum antibody levels during treatment with grass pollen-tyrosine adsorbate. *Clin. Allergy* 4, 57–70, 1974.
 4. Juul Sørensen, H.: Den kliniske værdi af hyposensibilisering ved asthma bronchiale og rhinitis allergica. *Ugeskr. Læg.* 138, 2703–2707, 1976.
 5. Levy, D. A., Lichtenstein, L. M., Goldstein, O. E. & Ishizaka, K.: Immunologic and cellular changes accompanying the therapy of pollen allergy. *J. Clin. Invest.* 50, 360–369, 1971.
 6. Lichtenstein, L. M., Ishizaka, K., Norman, P. S., Sobotka, A. K. & Hill, B. M.: IgE antibody measurements in ragweed hay fever. Relationship to clinical severity and the results of immunotherapy. *J. Clin. Invest.* 52, 472–482, 1973.
 7. Norn, S. & Stahl Skov, P.: Anaphylactic hyposensitization of rat mast cells in vitro by antigen. *Clin. Exp. Immunol.* 18, 431–437, 1974.
 8. Norn, S., Elmgreen, J., Stahl Skov, P., Holme Jørgensen, P., Ankjærgaard, N. & Hagen Petersen, S.: Influence of hyposensitization on ATP level and CO₂ production of mast cells in anaphylaxis. *Clin. Exp. Immunol.* 26, 162–168, 1976.
 9. Reisman, R. E., Wypych, J. I. & Arbesman, C. E.: Relationship of immunotherapy, seasonal pollen exposure and clinical response to serum concentration of total IgE and ragweed-specific IgE. *Int. Arch. Allergy Appl. Immunol.* 48, 721–730, 1975.
 10. Stahl Skov, P. & Norn, S.: A simplified method for measuring basophil histamine release and blocking antibodies in hay fever patients. Basophil histamine content and cell preservation. *Acta Allergol.* 32, 170–182, 1977.
 11. 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.
 12. Stahl Skov, P., Permin, H. & Malling, H.-J.: Quantitative and qualitative estimations of IgE bound to basophil leukocytes from hay fever patients. *Scand. J. Immunol.* 6, 1021–1028, 1977.
 13. Østerballe, O.: Immunotherapy in hay fever with two major allergens 10, 25 and partially purified extract of timothy grass pollen. A controlled double blind study. In vivo variables. Season I. *Allergy* 35, 173–189, 1980.

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