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IMMUNOLOGIC CHANGES DURING SPECIFIC TREATMENT OF THE ATOPIC STATE

I. Allergen Binding Capacity of Atopic Sera in the Course of Hyposensitization Treatment

BY

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The allergen binding capacity of atopic sera is a function not only of reaginic, but also of other types of antibodies (*Ishizaka, Ishizaka & Hornbrook 1966, Ishizaka, Ishizaka & Hornbrook 1967, Ishizaka, Ishizaka & Menzel 1967, Yagi, Pressman, Arbesman & Reisman 1963, Kopp, Bilbo & Reed 1965, Lidd & Farr 1962, Lidd & Connell 1964, Pruzansky, Paterson & Feinberg 1962, Pruzansky & Paterson 1964, Hale, Cawley, Holman & Miner 1967*). Using radioisotopic methods it was possible to follow the binding of antibody with antigen and to look for the relationship between the binding capacity and the clinical state of the patient. Up till now, however, these connections have not been investigated in a sufficient number of patients. In our study the attention was concentrated on following the allergen binding capacity of immunoglobulins G, A, M and D in course of the first and fifth year of treatment of hay fever.

MATERIAL AND METHODS

We investigated two different groups of hay fever patients: 30 untreated patients (RP₀) with high skin hypersensitivity to timothy allergen and 30 patients with the same hypersensitivity type after four hyposensitization treatments (RP₄). All sixty patients were aged between 15 and 35 years and of both sexes. Samples

of sera were drawn before the first, or the fifth treatment respectively, and ten days after the last injection. To all patients, 84,000 PNU of aqueous allergen Sevac (Prague) were given during four months of the preseasonal treatment. The evaluation of symptoms was judged by a point system according to *Connell & Sherman 1967*. Sixty points of symptoms and medication gave the limitation between mild and high severity of symptoms. The decrease of more than 25 per cent in number of points between the following season and the pretreatment state was a sign of clinical improvement. The skin sensitivity to the timothy allergen (1000 PNU) was investigated by the prick test and evaluated in two degrees (high and mild sensitivity) according to the diameter of the wheal (> 1 cm or < 1 cm).

All the sera were drawn on the same day, refrigerated until used at -40°C , and investigated with one batch of agar plates, antisera and allergen simultaneously by radioimmuno-electrophoresis (RIE) and radioimmunoplate method (RIP). The radioimmuno-electrophoretic technique and autoradiography were carried out according to Yagi's indirect method (*Yagi, Maier, Pressman, Arbesman & Reisman 1963*) with incubation time for 4 hours in I^{131} labelled timothy allergen. The capacity of binding with labelled allergen was expressed semi-quantitatively in three degrees: no or weak binding (—), definite binding (+) and strong binding (++) .

For radioimmunoplate technique the immunoglobulin contents were followed by single radial immunodiffusion (*Fahy & McKelvey 1965*), using monospecific antisera and standards Sevac (*Škvařil & Rádl 1969*). After washing the plates were incubated in the same timothy extract as in radioimmuno-electrophoresis for four hours. After repeated washing lasting for three days the radioactivity of every precipitate in the same volume of agar was measured in a well type scintillation counter for 3 minutes.

As antigen, a partially purified timothy extract was used after separation from low molecular components by means of filtration through Sephadex G-25 column and dialysis (*Stajner & Zuváczal 1969*). In skin tests its allergen activity was ten times higher than the activity of the corresponding crude extract Sevac which was used for hyposensitization treatment. The antigen was labelled with I^{131} according to Yagi's technique (*Yagi, Mair, Pressman, Arbesman & Reisman 1963*) to the final activity of 0.6 mc/mg. The concentration of allergen used was 16 mg/ml.

For the statistical evaluation the χ^2 test or Smirnov-Kolmogorov test with critical values of $z = 1.37$ ($P = 5$ per cent) and $z = 1.64$ ($P = 1$ per cent) were used.

RESULTS

The first figure presents a typical example of different binding capacity of immunoglobulins G, A and M in radioimmuno-electrophoresis. Between the serum samples before and after

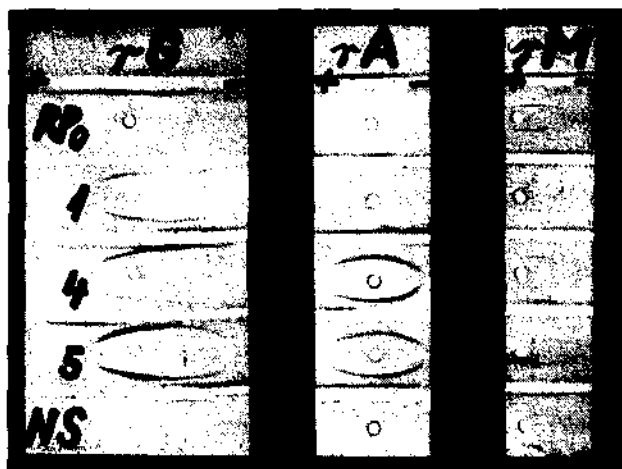


Fig. 1.

Allergen binding activity of serum immunoglobulins G, A and M in radio-immuno-electrophoresis with I¹²⁵ labelled timothy allergen.

Wells: RP₀ = serum of an untreated patient; RP₁ = the same serum after finished the 1st treatment; RP₄ = serum of a patient before the 5th treatment; RP₅ = serum of the same patient after finished the 5th treatment; NS = normal serum.

Troughs: monospecific sera against γG, γA and γM.

the first or the fifth treatment respectively, an increase in radioactivity of IgG was very regular. A clear difference was observed between the radioactivity of untreated, once treated and repeatedly treated cases and between the binding activity of patients and negative binding of healthy persons. It is interesting that the maximum of IgG binding activity was always concentrated in the fast fraction of this immunoglobulin in electrophoresis. A similar increase of binding activity was also seen in IgA, but the differences were smaller and irregular. For IgM only a weak binding activity was noted and its increase was very rare, whereas no binding capacity was observed in IgD.

All results in radioimmuno-electrophoresis are summarized in the next graph. A considerable and statistically significant increase of IgG binding activity during the treatment was

stated in patients of both groups ($\chi^2 = 9,623$, $P \leq 1$ per cent for 1st treatment and $\chi^2 = 16,172$, $P \leq 1$ per cent for repeated treatment). The IgG binding activity of sera in the case of the first treatment was significantly lower than in patients after repeated treatment ($\chi^2 = 17,575$, $P \leq 1$ per cent for pretreatment samples, $\chi^2 = 10,980$, $P \leq 1$ per cent for post-treatment sera). A similar but weak increase in binding capacity of IgA and IgM was statistically not significant.

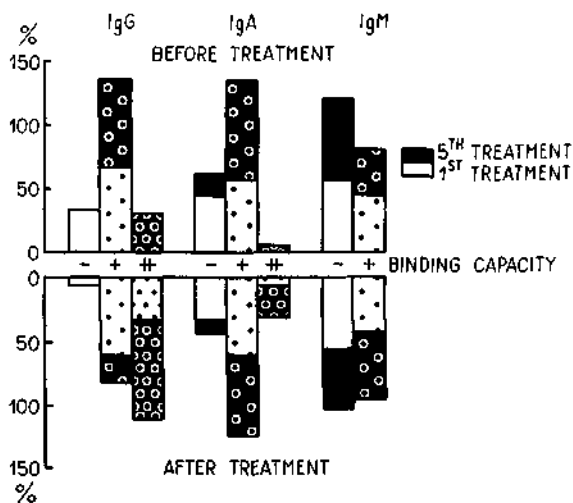


Fig. 2.

Allergen binding capacity of hay fever sera in the course of the 1st and the 5th treatment.

Some relations between the binding capacity of sera and the clinical symptoms of hay fever were followed in the next pollen season. From the graph it is evident that the patients with higher binding activity already before treatment had, in the following season, rather fewer symptoms than patients with weak binding capacity. This statement is evident for all types of immunoglobulins, especially for IgG. The same relation was noted also for post-treatment sera.

Also the "total binding activity" of sera, taken by the sum of activities of IgG, A and M, was in a similar relation to the

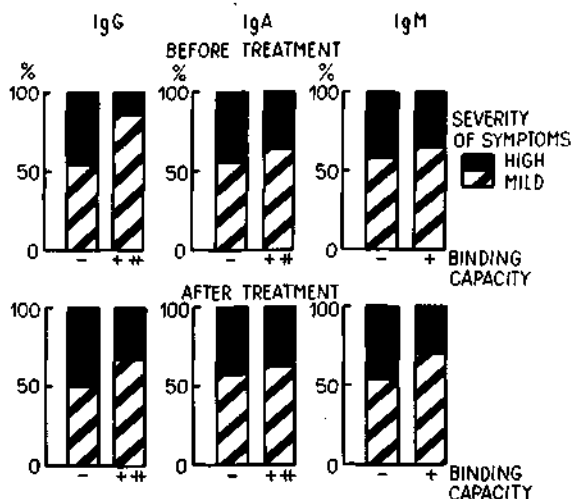


Fig. 3.

Relationship between the allergen binding capacity of hay fever sera and the severity of symptoms in patients during the next pollen season.

clinical state. In the group of patients with higher total binding capacity seventy per cent of all were better in the pollen season in comparison with thirty per cent of cases with a weak binding activity. This difference was statistically significant ($\chi^2 = 3,853$, $P \leq 5$ per cent). The treatment being finished, the observations had the same character, the binding activity was, however, higher on the whole ($\chi^2 = 2,355$).

Also in the evaluation of the improvement of the disease a higher effect of treatment was observed in those patients, where the binding activity was stronger.

Very similar results were observed using the radioimmuno-plate method which made possible the evaluation of binding activity using scintillation counting. A direct and significant relation of number of impulses to the semiquantitative evaluation of binding activity in radioimmuno-electrophoresis was noted ($\chi^2 = 4,602$, $P \leq 5$ per cent). In the next graph the results are recorded, referring to the group of patients before the first treatment and after its finishing in comparison with

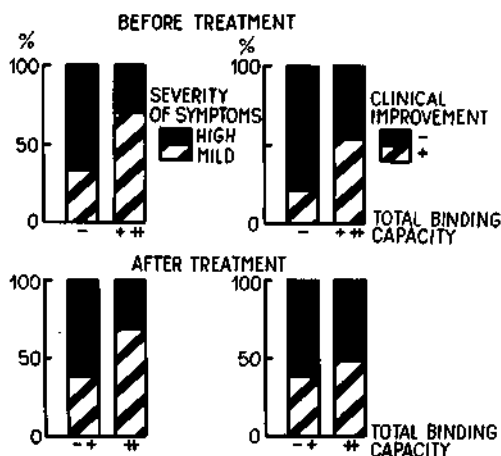


Fig. 4.

Relationships between the "total allergen binding capacity" of hay fever sera and the severity of symptoms or clinical improvement in patients during the next pollen season.

the group of repeatedly treated patients after the last hypo-sensitization. Also here a striking increase of binding activity, especially of IgG, was found in relation to the number of treatments. Patients with higher number of impulses were rather better and their improvement of the disease was more often seen than in the group of cases with a weak binding activity.

It is known that to a certain extent the clinical sensitivity may be expressed by the skin sensitivity of the patient. From this view-point an indirect relation between the binding activity and the grade of skin sensitivity might be expected. The following graph shows that patients with a higher total allergen binding activity of serum before and also after the treatment, react more often with a weak skin reaction to the same timothy allergen than the patients with the low binding capacity. These differences did not reach, however, the level of statistical significance ($\chi^2 = 1,132$ and $2,427$).

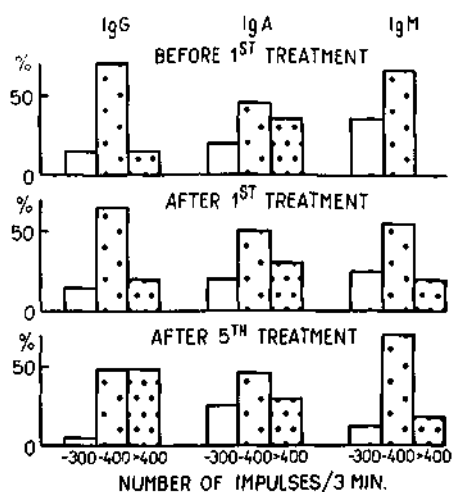


Fig. 5.

Allergen binding activity in numbers of impulses in the course of hay fever specific treatment.

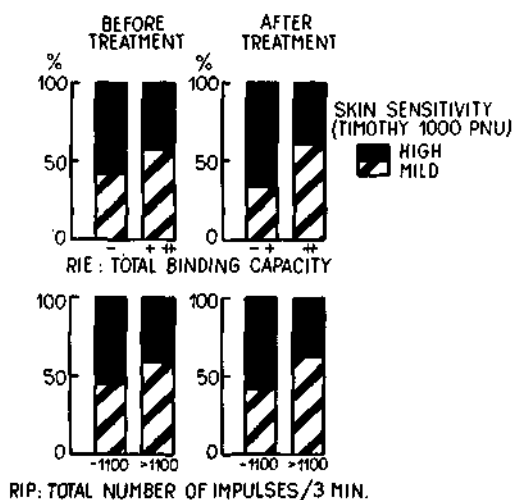


Fig. 6.

Allergen binding activity and skin sensitivity in the course of hay fever specific treatment.

RIE = radioimmuno-electrophoresis; RIP = radioimmunoplate technique; high skin sensitivity = diameter of wheal > 1 cm; mild skin sensitivity = diameter of wheal < 1 cm.

DISCUSSION

Our results show a certain indirect relation between the clinical, or skin sensitivity of hay fever patients and the allergen binding activity of their sera, or of immunoglobulins G, A and M respectively. These relations are very regular, but not so distinct to be in every case statistically significant in the limited number of simultaneously investigated patients and antigens. Our results are in accordance with the experience of *Ishizaka*, *Ishizaka & Hornbrook* 1967, that all types of antibodies binding with antigen may have a blocking effect by interference with allergen. From this view-point immunoglobulin G because of its highest serum level seems to be the most important, and also immunoglobulin A for its possible role in the resistance of respiratory mucous membrane. Similar results were obtained by *Goodfriend*, *Perelmutter*, *Phills & Rose* 1966 and *Goodfriend*, *Phills & Perelmutter* 1969 in the investigation of the blocking activity of different immunoglobulin fractions. Also the different blocking activity of IgG molecules of different electrophoretic mobility was in agreement with our results. *Lichtenstein* 1968 and *Lichtenstein*, *Holtzman & Burnett* 1968 using the inhibition of antigen E induced histamine release from human leukocytes, observed a good correlation of blocking activity, pollen tolerance and chromatographic elution of the IgG molecules. The latest paper of *Spitzer*, *Kaplan & Leija* 1968 emphasizes the role of IgG molecules in the allergen neutralizing activity, too. Our results are in agreement with all these experiences that the most significant changes in course of repeated hyposensitization appear in IgG, less in IgA, while the binding capacity and dynamics of IgM and IgD have no definite relations to the clinical improvement. But though these relations between the clinical sensitivity and the allergen binding capacity of sera are very regular, it is not possible to suppose that the increase in binding activity of serum immunoglobulins is the only mechanism of the improvement. Also other changes, for example in the contents of reaginic antibodies (*Connell & Sherman* 1967) and in the

complex of nonspecific regulations (*Burdon 1967, Zavázal 1969*) may take part in the favourable effect of hyposensitization treatment.

SUMMARY

The authors followed the allergen binding activity of sera in 60 hay fever patients during the first and the fifth hyposensitization treatment by means of radioimmuno-electrophoretic and radioimmunoplate technique. In both methods an increase of antigen binding activity was observed during the course of treatment. The greatest increase was noted in IgG, especially in the fast part of the electrophoretic mobility. This increase in antigen binding capacity of post-treatment sera was to a certain extent directly proportional to the decrease of the clinical and skin sensitivity of patients.

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