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IMMUNOLOGIC CHANGES DURING SPECIFIC TREATMENT OF THE ATOPIC STATE

III. *The Immunologic Response in the Course of Hyposensitization of Hay Fever Patients with Aqueous and Alum - Pyridine Allergens*

By

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A comparable effect of hyposensitization treatment in hay fever patients with aqueous and repository allergens has been noted by many authors. The repository technique, however, offered a great saving of time and reduced the risk of overdose reactions, even when a lightly better clinical effect was seen in our patients treated with aqueous extracts (Zavázal & Nová 1964). Lichtenstein, Norman & Winkenwerder 1968 compared the clinical and immunological response of both types of allergens: The group of patients treated with aqueous extract had a significantly greater antibody response than a similar group treated with Allpyral. In this report the immunoglobulin levels and allergen binding activities were followed during the fifth treatment with both procedures in selected persons with similar original hypersensitivity.

MATERIAL AND METHODS

Two groups of hay fever patients treated four years either with aqueous or alum-pyridine allergens were compared. Before treatment these groups not only had the same length of history of the disease, but also the same type of skin sensitivity and very similar severity and character of symptoms. All 49 persons were in the age groups between 15 and 35 years and were of both sexes.

Both groups were followed during their fifth therapy. The group of 21 pa-

tients, treated with native grass pollen allergen Sevac (group RP_N) received 36 injections during 4 months of treatment and a total dose of 84 000 PNU. The 28 patients in the second group (RP_D) received 80 000 PNU of the alum-pyridine grass allergen in 4 preseasonal injections. According to the producer's reference this allergen was extracted with pyridine, precipitated by zinc chloride and tannic acid, formolized and adsorbed on $Al(OH)_3$. Both types of allergens were prepared and supplied by Dr. J. Liška, Sevac-Prague. The evaluation of symptoms in the next pollen season was carried out by the point system of Connel & Sherman 1967. Sixty points of symptoms and medication gave the limitation between the mild and high severity of hay fever symptoms after the fifth treatment. The skin sensitivity to the timothy allergen (1 000 PNU) was investigated by the prick test and evaluated in 3 degrees (negative, mild, high sensitivity) according to the diameter of the wheal (< 4 mm, < 10 mm, > 10 mm).

The sera of both groups were taken at the same time and refrigerated at a temperature of -40° C. Samples of sera were drawn shortly before the 5th hyposensitization treatment, then 5 weeks later after reaching the dose of 275 PNU (group RP_N), or 2 000 PNU (RP_D) and 10, or 20 days after the last injection of native, or alum-pyridine extract respectively, which is shortly before the beginning of the pollen season.

The immunoglobulin content was determined by immunoplate technique on large plates for 60 samples (Fahey & McKelvey 1965) using monospecific antisera and standards Sevac (Prague) (Škvařil & Rádl 1969). The simultaneous investigation of all 3 samples of both groups facilitated the exact evaluation of the results. The binding activity of immunoglobulins for labelled timothy allergen was investigated according to the radioimmunoelectrophoretic method of Yagi, Maier, Pressman, Arbesman & Reisman 1963 as mentioned in the foregoing paper (Zavázal & Stajner 1969). The capacity of the allergen binding was expressed in five degrees according to the intensity of radioactivity: none of a weak binding as negative, definite binding as 1 + to 4 +.

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

RESULTS

I. Differences in Clinical and skin sensitivity after Five Years of Therapy with Aqueous and Alum-Pyridine Allergens

From the two graphs it may be observed that not only the clinical sensitivity, but also—and in this case significantly ($\chi^2 = 10.346$)—the skin sensitivity decreases in the case of aqueous allergen therapy more remarkably than after slow acting allergen suspensions.

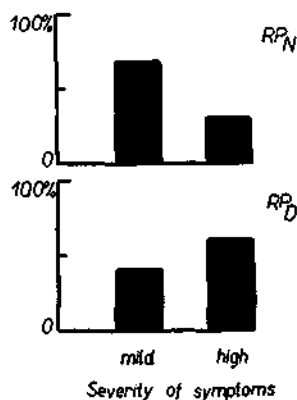


Fig. 1.

Severity of symptoms hay fever patients treated for five years with native (RP_N) and alum-pyridine (RP_D) allergens Sevac-Prague.

II. Differences in Immunoglobulin levels in the Course of the Fifth Treatment with Native and Alum-Pyridine Allergens

Shortly before the 5th therapy the IgG contents in patients treated with alum-pyridine allergens was not significantly

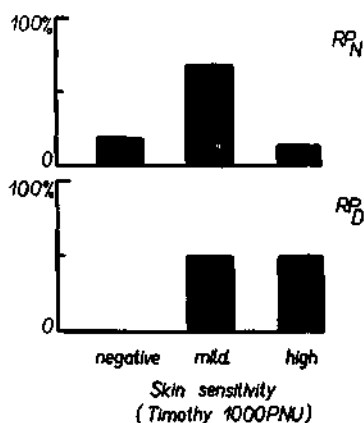


Fig. 2.

Skin sensitivity in hay fever patients, treated with native (RP_N) and alum-pyridine (RP_D) allergen for five years. Mild sensitivity: wheal diameter: 4 to 10 mm. High sensitivity: wheal diameter: > 10 mm.

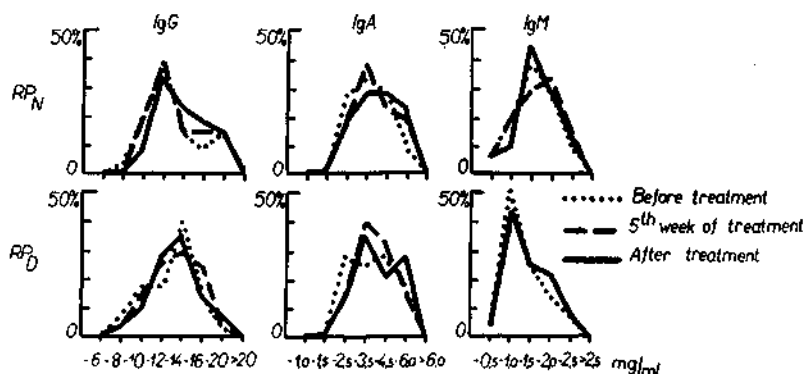


Fig. 3.

Immunoglobulin levels during the fifth hay fever treatment with native (RP_N) and alum-pyridine (RP_D) allergens.

higher, perhaps in consequence with the last injection of allergen before 4 months and its protracted immunologic effect. On the contrary, the contents of immunoglobulin M was not significantly lower in comparison with the native group. During the 6 weeks of treatment the immunoglobulin levels increased slightly, especially in IgM after native therapy and at the end of hyposensitization the immunoglobulins G and A reached nearly the same levels in both groups of patients. Only the IgM levels remained lower and almost without changes in patients treated with alum-pyridine allergens.

III. Differences in Allergen Binding Capacity of Sera during the Fifth treatment with Aqueous and Alum-pyridine Allergens

The allergen binding activity was measured before and after finishing the 5th treatment. In radioimmuno-electrophoresis with I^{131} timothy allergen a lower binding capacity was noted in the repository group for γG and γM immunoglobulins before the 5th treatment. Also after the treatment the binding activity of IgG increased more significantly in patients, treated with aqueous extracts. This difference between both groups of patients was highly significant ($z = 1.83$). In

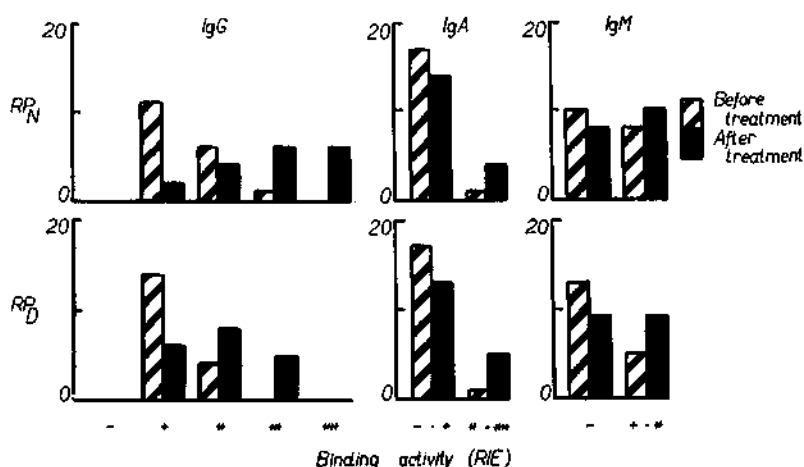


Fig. 4.

Allergen binding activity in radioimmuno-electrophoresis (RIE) in course of the 5th hay fever treatment with native (RP_N) and alum-pyridine (RP_D) allergens.

immunoglobulins A and M this difference between both groups of patients was not significant, but also a slight increase of the binding capacity during the fifth treatment, especially in IgM, could be observed.

A certain relation was noted between the binding activity and the clinical tolerance of pollen allergen in the next season or in skin tests. The higher binding activity in patients treated with aqueous extracts was often connected with a better clinical condition and milder skin sensitivity while the lower binding capacity of sera after treatment with alum-pyridine allergens were seen rather in cases with higher severity of symptoms and greater skin hypersensitivity.

DISCUSSION

It was shown that there are certain differences not only in the effect of the repeated therapy with aqueous or alum-pyridine allergens, but also in the immunologic response of both groups of patients. A similar statement was observed by inhibition of

histamine release from sensitive leukocytes after challenging with ragweed allergen by *Lichtenstein, Norman & Winkler* 1968. Also using indirect haemagglutination method some differences could be observed (*Reisman & Arbesman* 1965, *Spiegelman, Friedman & Tuft* 1967 and *Zavázal & Stajner* 1969) in comparison with the native treatment. Before the last treatment with alum-pyridine allergens the immunoglobulin G levels were slightly higher, but these differences diminished in the course of the fifth hyposensitization. At the end of this treatment it was possible to observe only a higher content of IgM in patients with aqueous therapy. It is, however, rather interesting that in the alum-pyridine group (RP) the allergen binding activity of IgG remains significantly lower although its total serum level was not lower on the whole. This smaller allergen binding activity was connected with higher severity of clinical symptoms and higher skin sensitivity to the same timothy allergen in this group. It may be supposed that this immunological difference takes part in the different therapeutic effect of both allergens. At present it is not possible to decide whether the lower binding capacity after the repository therapy is caused by a higher production of nonspecific globulins or of antibodies of a weaker allergen binding activity and blocking activity. It is, however, necessary to consider also other specific and non-specific differences between two groups of patients. More extensive studies in the future may help to explain the mechanism of both types of hyposensitization treatment, which remains still unexplained.

SUMMARY

The serum levels of immunoglobulins G, A and M and the allergen binding capacity of sera were investigated in hay fever patients treated repeatedly with aqueous or alum-pyridine pollen allergens. The higher pollen tolerance of patients treated with native allergens was seen with the higher binding activity of IgG, even when its serum level was nearly the same

in both groups. This difference in the immunologic response, may be in certain relation to the different effect of both types of therapy.

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