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A METHOD FOR DEMONSTRATION OF BLOCKING ANTIBODIES IN DESENSITIZED HAY FEVER PATIENTS*

By

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Several mechanisms have been thought to be responsible for the alleviating effect of desensitization treatment in allergic patients: induction of partial tolerance, whether or not caused by diminution of the level of reagins, formation of blocking antibodies, etc. In this paper we shall discuss the blocking antibodies.

Passive transfer experiments done by *Cooke et al.* (1) and by *Loveless* (5) led to the conclusion that in the sera of desensitized patients a second, heat stable antibody had been developed. This antibody was called blocking antibody after its effect in the method used. The disadvantages of the passive transfer technique were avoided by *Maunsell* (6), who tested the patients on their own skin with two dilution series of pollen extract, one mixed with saline and the other with serum in a constant amount of 50 per cent. Before treatment, no difference was seen between the two series. After treatment, the threshold skin reaction in the pollen-serum series was in response to a higher pollen concentration than that in the pollen-saline series. The ratio of the threshold values in

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the two series was called the blocking titre. However, skin reactions do not always decrease gradually when a dilution series is tested, and the 50 per cent serum dilution may give rise to non-specific reactions. Consequently, *Voorhorst* (7) proposed another technique in which the amount of pollen extract was kept constant and a dilution series of the serum was applied. Furthermore, the serum was heated before use, so that the reagins were inactivated.

MATERIAL AND METHOD

The method of *Voorhorst* (7) to assess the titre of blocking antibodies on the patient's own skin was used in 107 untreated and desensitized hay fever patients. The details of this method are as follows:

1. Serum (containing reagins and blocking antibodies) is heated at 56°C for 30 minutes; the Fc-fragment of the reagins is thus inactivated.
2. Serum dilutions are prepared with final concentrations of 1/5, 1/15, 1/50, 1/150, 1/500, and 1/1500.
3. Equal amounts of allergen extract with final concentrations of 3 Noon U or 0.3 Noon U per ml are added.
4. Two controls are prepared: serum 1/5 without allergen, and allergen extract, 3 U or 0.3 U per ml, without serum.
5. The mixtures (and the controls) are kept at 37° C for 30 minutes to allow the reaction between allergen and blocking antibodies to occur.
6. Intracutaneous skin tests are performed with the mixtures and the controls.
7. The skin reactions are recorded after 20 minutes.

The titre lies where the serum dilution causes a distinct decrease of the skin reaction as compared with the control reaction of the allergen extract without serum. When there is no blocking effect at all, skin reactions are equal to the reaction with pollen extract alone. None of the patients showed any skin reaction to serum 1/5 without allergen extract.

RESULTS

The results of the investigations are given in Fig. 1. The patients are divided into five groups according to the duration of (perennial) treatment. On the vertical axis the titre of the blocking antibodies to 3 and 0.3 Units of grass pollen is indicated. The figures in the columns indicate the number of patients in whom a certain titre was found. At the top of

the columns the number of patients who showed no reaction whatsoever to 3 or 0.3 U of pollen is indicated.

The patients who were not treated showed no blocking antibodies with respect to 3 U of pollen; however, three patients produced a blocking effect with respect to 0.3 U.

DISCUSSION

The results obtained during treatment indicate that the longer the patients are treated, the higher the titres tend to become. One would expect that the titre related to 0.3 U would be ten times higher than that related to 3 U. That this is not so may be explained by the fact that skin reactions to 0.3 U pollen are rather small, which means that differences necessary for assessment of the titre are not always sufficiently visible. The number of non-reactors in Fig. 1 rises (at least

	31 patients		29 patients		19 patients		10 patients		16 patients	
	3 U	0,3 U	3 U	0,3 U	3 U	0,3 U	3 U	0,3 U	3 U	0,3 U
no skin reaction		4	5	13	5	11	1	7	2	11
blocking antibody titre										
1/1500										
1/500								1		1
1/150				2		3	3	1	5	4
1/50			1	5	3	4	4		6	
1/15		1	16	5	9		2	1	5	2
1/5		2	4	3	1	1				
0	31	24	3	1	1					
	-		< 1 yr.		1 - 2 yrs.		2 - 4 yrs.		4 - 8 yrs.	
	duration of treatment									

Fig. 1.

Blocking antibody titre in 107 untreated and desensitized hay fever patients.

to 0.3 U) according to the duration of treatment, as could be expected. In connection with the presence of blocking antibodies in three untreated patients it should be recalled that among other authors *Lichtenstein et al.* (1966) found that a low titre of blocking antibodies may also be present before treatment.

The method had some disadvantages. If one chooses too high a concentration of allergen extract, the influence of the blocking antibodies will not be visible, and if one chooses a concentration that is too low, there is no skin reaction at all and the blocking effect cannot be assessed. Another questionable point is the possible blocking effect of heated reagins, because the allergen-combining activity is only partially lost by heating (*Ishizaka et al.* (2)). However, since the amount of specific IgE is probably only a fraction of the amount of specific IgG, the former will have little influence on the results. To demonstrate this, four untreated patients—not recorded in Fig. 1—were tested by means of the *Maunsell* technique, using a constant amount of heated serum 1/5 or saline and a dilution series of the pollen extract. No differences were seen between the series, which suggests that the allergen-binding activity of heated reagins does not play a major role in the method used in this study. Moreover, *Lichtenstein et al.* (4) calculated that the binding activity of reagins in their treated patients probably amounts to well under 1 per cent of the total serum blocking activity.

As a result of desensitization, skin reactions decrease. By comparing skin reactions to three tenfold dilutions of the test extract in groups of patients, the average decrease of these reactions can be calculated. In 49 out of 76 treated patients

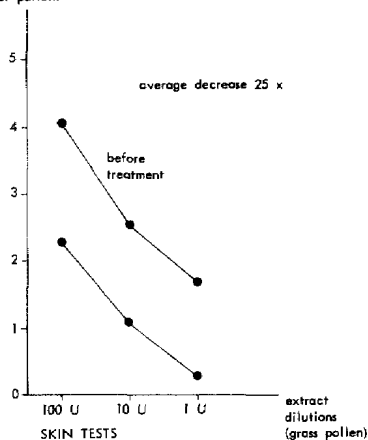
Fig. 2.

Decrease in skin reactions and blocking antibody titre in desensitization over a period of less than one year (mean 9 months).

Fig. 3.

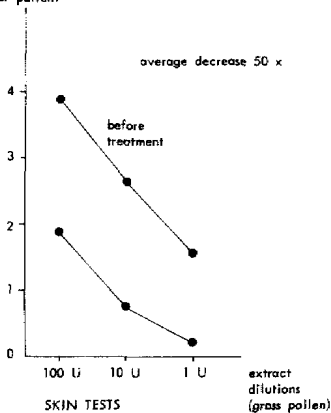
Decrease in skin reactions and blocking antibody titre in desensitization over a period of 1-2 years (mean 1 year and 6 months).

Duration of treatment < 1 year (15 patients)

average number
of plus signs
per patient

		3 U
blocking antibody titre	no skin reaction	2
	1/150	
	1/50	
	1/15	8
	1/5	3
	0	2

Duration of treatment 1 - 2 years (20 patients).

average number
of plus signs
per patient

		3U
blocking antibody titre	no skin reaction	6
	1/150	
	1/50	3
	1/15	8
	1/5	1
	0	

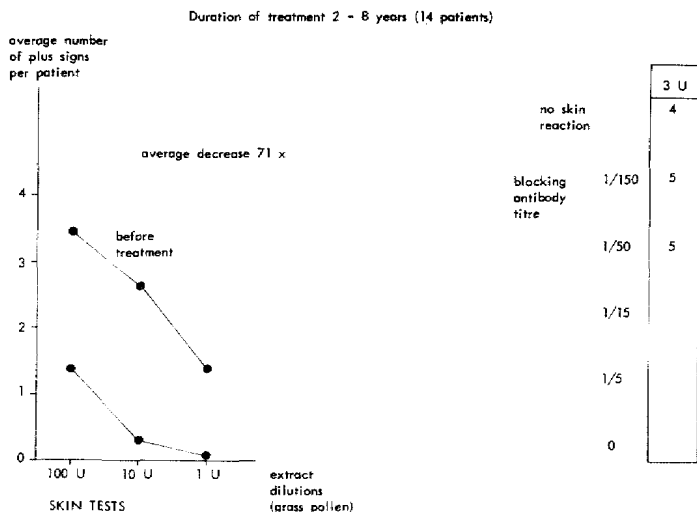


Fig. 4.

Decrease in skin reactions and blocking antibody titre in desensitization over a period of 2-8 years (mean 4 years and 4 months).

skin tests were done simultaneously with the assessment of the blocking antibody titre. This was a retrospective study in the sense that the skin reactions before treatment were not recorded as such but were expressed as a number of plusses in the patient's charts, so that exact data are not available for comparison. It should also be noted that the results do not concern a random sample of patients. Nevertheless, these results are shown, because a certain trend can be distinguished. Figs. 2, 3, and 4 show the average decrease of the skin reactions and the blocking antibody titres in the same group of patients. It is evident that during treatment the skin reactions become weaker and the blocking antibody titre becomes higher. This raises the question of whether, and if so to what extent, the decrease in the skin reactions is determined by the presence of blocking antibodies.

SUMMARY

Blocking antibody titres were assessed by skin-testing of hay fever patients with mixtures consisting of a constant amount of pollen extract and a dilution series of heated serum. The untreated patients had on the whole no titre. The titres in desensitized patients tended to become higher the longer the patient was treated.

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