

THE SPECIFICITY OF THE HUMORAL FACTORS IN TRANSPLANTATIONAL IMMUNITY

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Problems concerned with the part played by humoral factors of the recipient in transplantational immunity during homografting are of great importance to the discovery of the mechanisms of tissue incompatibility.

During recent years papers have appeared in the literature demonstrating the formation of immune antibodies (hemagglutinins) in the recipient after homografting of dogs' kidneys [6, 7] and of the skin of mice [3, 5]. However, the properties of the antibodies formed during homografting have received little study. According to some workers, the changes in the agglutinating properties of the recipients' serum during homografting may even be of the nature of nonspecific humoral changes [4]. In previous investigations [1, 2] we showed that in the majority of rabbits after homografting of skin immune antibodies are formed, whereas after autotransplantation no antibodies appeared.

The present investigation is devoted to the study of the specificity of the antibodies formed during homotransplantation of the skin in rabbits. We carried out serological investigations on the sera of 19 recipient rabbits containing immune antibodies after homotransplantation.

EXPERIMENTAL METHOD

The properties of the immune sera were studied by means of the hemagglutination and adsorption reactions. The hemagglutination reaction was performed at room temperature, and in a volume of 2 drops in a tube, with subsequent centrifugation for 1 minute at 1500 rpm. The results were read according to the character of the agglutinate. Adsorption was carried out at room temperature with a precipitate of triple-washed red cells in a proportion of 1:1. The serum was first diluted half-and-half with physiological saline.

EXPERIMENTAL RESULTS

Investigation of the rabbit sera before and after grafting with chick's and sheep's red cells showed that the sera of recipients after homotransplantation had not altered their agglutinating properties in relation to heterogenic red cells. The sera of the recipients acquired the property of agglutinating only the red cells of rabbits, i. e. they had the nature of immune iso-antibodies. Immune sera from the recipients were tested with red cells of a large number (60) of the same rabbits.

Table 1 shows the results of the investigation by means of the hemagglutination reaction of the sera of 15 recipient rabbits with red cells of 25 rabbits. In the vertical column on the left are placed the identity numbers of the recipient rabbits whose sera were being examined. In the top horizontal column are shown the identity numbers of the rabbits with whose red cells the examination of the sera was being carried out. A positive hemagglutination reaction is represented as +, a negative -, and doubtful results as ±. The results of the test of the serum with the red cells of the donor and recipient are marked with a ring.

TABLE 1

[illegible]

TABLE 2

Properties of the Hemagglutinins of Rabbit No. 1236 after Homotransplantation of Skin

No. of rabbits whose red cells were used for adsorption	No. of rabbits whose red cells were used in agglutination	Dilution of serum						Control red cells
		1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	
14	1717	+	—	—	—	—	—	—
	812	+	—	—	—	—	—	—
	14	+	—	—	—	—	—	—
	756	±	—	—	—	—	—	—
	1717	++	++	++	++	+(+)	+	—
866	812	++	++	++	++	+	—	—
	14	++	++	++	+	+	—	—
	756	++	++	++	+(+)	+	—	—
	1717	+	±	—	—	—	—	—
	812	+	—	—	—	—	—	—
756	14	±	—	—	—	—	—	—
	756	—	—	—	—	—	—	—
	1717	++	++	++	++	+(+)	+	—
	812	++	++	++	++	+	—	—
	14	++	++	++	+	+	—	—
Serum before adsorption	756	++	++	++	+(+)	+	—	—
	866	—	—	—	—	—	—	—
	786	—	—	—	—	—	—	—

The serum of each recipient rabbit always gave a positive reaction with the red cells of its donor, but did not agglutinate its own red cells. In addition the recipients' sera agglutinated the red cells of other rabbits, but not all with which they were tested. Thus the serum of recipient No. 1236 agglutinated, besides the red cells of its donor (rabbit No. 1717), also the red cells of rabbits Nos. 67, 756, 46, 812, 14, 811, 829, 603, 365 and 333. The ability of the recipient's serum to agglutinate the red cells of the rabbits listed evidently developed as a result of the presence in these rabbits of common antigens with the donor that were absent from the recipient. It is clear that it is only to this form of antigen in the donor that antibodies could appear in the recipient.

At the same time the recipient's serum did not agglutinate the red cells of rabbits Nos. 866, 786, 442, 407, 818, 510, 813, 77 and 71. The negative reaction was evidently the result of the fact that the rabbits listed had no common antigens with the donor against which antibodies could appear in the recipient. On the basis of their similarity of behavior in relation to the sera of the 15 recipients, the rabbits' red cells may be arranged in several groups.

In the 1st group we placed rabbits Nos. 866, 407, 786, 643, 701, 510 and 292, whose red cells were not as a rule agglutinated by the immune sera of 8 recipient rabbits (Nos. 866, 510, 407, 292, 77, 1236, 177 and 71). However, they gave a positive reaction with the sera of 7 other recipient rabbits (Nos. 812, 811, 756, 818, 442, 1717 and 786). Despite their great similarity, there were also differences between the rabbits of this group. Rabbits Nos. 866 and 407, for instance, differed from the others by the fact that their red cells gave a positive reaction with the serum of recipient rabbit No. 292. Rabbits Nos. 786, 643 and 292 differed from the others by the fact that their red cells were not agglutinated by sera of recipient rabbits Nos. 1717 and 786. At the same time rabbit No. 292 differed from the other rabbits mentioned above by the fact that the sera of recipient rabbits Nos. 866 and 71 agglutinated its red cells.

In the 2nd group we included rabbits Nos. 1236, 71, 818, 177, 77, 442, 813 and 333. The red cells of these rabbits as a rule were not agglutinated by the serum of the 4 recipient rabbits Nos. 77, 1236, 177 and 71.

The sera of the other recipients agglutinated the red cells of these rabbits. Among the rabbits of the second group, as among the animals of the preceding group, despite their similarity, differences were also to be found. For instance rabbit No. 333 differed from the others by the fact that its red cells were agglutinated by the serum of recipient rabbit No. 1236. Rabbit No. 818 differed by the fact that its red cells were agglutinated by the serum of recipient rabbit No. 177. The red cells of rabbits Nos. 77 and 442, which were very similar, behaved differently to the serum of rabbit No. 866 and so on. In a similar manner we distinguished a 3rd and 4th group of rabbits.

Consequently in each group we included rabbits whose red cells behaved in a similar manner in relation to the immune sera of the 15 recipient rabbits, but often there were differences between them. In this way the differences between the rabbits within one group were smaller than those between rabbits belonging to different groups.

The properties of the sera of the recipient rabbits in relation to the red cells of different rabbits were also characterized by features of similarity and dissimilarity. On the basis of the similarity of the properties of the sera in relation to red cells, they could be seen to fall into several categories. In category I were placed recipient rabbits Nos. 866, 510, 407 and 292, whose sera agglutinated the red cells of rabbits of the 2nd, 3rd and 4th groups. However, the sera of the recipients in category I did not agglutinate the red cells of the rabbits in the 1st group. This evidently was because the recipients themselves were members of the 1st group of rabbits. The similarity of the properties of the sera of the recipient rabbits in category I was probably also due to the fact that their donors were rabbits Nos. 67, 46 and 603, which were very similar in an antigenic respect and belonged, as is seen from Table 1, to group 4 of animals. The red cells of one donor (rabbit No. 818), it is true, belonged to the 2nd group. In spite of their great similarity, between the sera of the recipients in category I there were also differences. For instance, the serum of recipient No. 292, in contrast to the others, agglutinated the red cells of rabbits Nos. 866 and 407. The serum of rabbits No. 866 in turn, did not agglutinate the red cells of rabbits Nos. 77 and 813. The sera of recipient rabbits Nos. 510 and 407 showed no differences in relation to the red cells of the 25 rabbits, but when tested with red cells of a larger number of rabbits it was possible to establish differences between them in relation to the red cells of 2 groups of rabbits.

In category II were included recipient rabbits Nos. 77, 1236, 177 and 71, whose sera agglutinated the red cells of rabbits of the 3rd and 4th groups. However, they did not agglutinate the red cells of rabbits of the 1st and 2nd groups. This was evidently because the recipients themselves belonged to the 2nd group. They did not agglutinate the red cells of rabbits in group 1, probably because the rabbits of groups 1 and 2 had certain common antigens. The similarity of the properties of the sera of recipient rabbits in category II was also demonstrated by the fact that their donors (rabbits Nos. 14, 1717 and 829) had a great antigenic resemblance and they belonged to group 4. The red cells of one donor (rabbit No. 811) belonged to group 3. In spite of the fact that the sera of the category II recipients showed great similarity, there were also certain well-defined differences between them. Serum of recipient No. 71, for instance, in contrast to the others, agglutinated the red cells of rabbit No. 292. The serum of recipient rabbit No. 177 differed from the others by agglutinating the red cells of rabbit No. 818. The serum of recipient No. 1236, in contrast to the others, agglutinated the red cells of rabbit No. 333. In a like manner it was possible to distinguish categories III and IV of rabbits.

Consequently the properties of the recipients' sera in relation to rabbits' red cells demonstrate the presence therein of common and different antigenic components. In spite of the similarity in the properties of the sera, we were unable to find any one pair of recipients whose sera would behave in the same way towards the red cells of a large number of rabbits; there were always greater or smaller differences between them. These findings demonstrate that the antigenic structure of the animals examined varies considerably. The presence in these rabbits of common antigenic components, responsible for the similarity in the properties of the red cells, is also confirmed by the results of the tests of the adsorptive properties of the red cells in relation to the immune sera of the recipients.

In Table 2 are given the results of the investigation of the serum of rabbit No. 1236 by means of the hemagglutination reaction before and after its adsorption with the red cells of 3 rabbits - Nos. 14, 866 and 756.

The red cells of rabbits Nos. 14 and 756, which gave a positive reaction with the serum of the recipient before adsorption, possessed the property of adsorption of antibodies from it. This can be seen from the fact that the serum of recipient No. 1236, after adsorption by red cells of rabbits Nos. 14 and 756, lost the power of ag-

glutinating rabbits' red cells. The red cells of rabbit No. 866, which reacted negatively with the test serum before adsorption, were incapable of adsorbing antibodies from it. The serum of rabbit No. 1236, after adsorption by the red cells of rabbit No. 866, did not lose its agglutinating properties in respect to the red cells of the donor (rabbit No. 1717) and of other rabbits (Nos. 14, 812, 756). The immune sera of other recipient rabbits behaved in precisely the same way after adsorption by the red cells of different rabbits. Thus rabbits, in spite of their considerable similarity antigenically, nevertheless show great variety. The antigenic differences between rabbits may be revealed during homografting by the formation of specific iso-antibodies in the recipient. The antigenic similarity of rabbits is presumably responsible for the agglutination of red cells of other rabbits as well as those of the donor by the immune serum of the recipient.

The results of the hemagglutination reaction and also of the adsorption of the sera by red cells show that the humoral changes arising in recipient rabbits during homoplastic skin grafting have the nature of specific immune iso-antibodies. A study of the antigenic structure of rabbits, carried out by means of immune sera of recipients, showed the existence in rabbits of wide antigenic differences, which evidently lie at the basis of their biological incompatibility. Cross-testing of red cells and immune sera led to the segregation of the rabbits into several groups on the basis of the greater or lesser antigenic similarity between them.

SUMMARY

An investigation was carried out on immune sera obtained from 19 rabbit-recipients of skin homotransplants. The reaction of hemagglutination of these sera with erythrocytes of a large number (60) of rabbits was tested. On the grounds of the similarity of behavior of erythrocytes towards the immune sera, and of the immune sera with respect to the erythrocytes, the rabbits could be subdivided into several groups. However, there were antigenic differences between the rabbits even within a group. The antigenic similarities and differences of the rabbits were confirmed by the reaction of adsorption of the sera by the erythrocytes. The data obtained in these experiments make it possible to assume that the humoral changes produced as a result of skin transplantation are of the specific immune iso-antibody nature.

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* See English translation.