EXPERIMENTAL BIOLOGY

CHANGES IN THE IMMUNOLOGICAL PROPERTIES OF RABBIT SERUM FOLLOWING HOMOLOGOUS SKIN GRAFTS

E. A. Zotikov

From the Laboratory of Biological Antigens (Director – Prof. P. N. Kosyakov) Institute of Experimental Biology (Director – Prof. I. N. Maisky) Acad. Med. Sci. USSR, Moscow

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As is well known, homotransplants of skin, as a rule, do not take, whereas autotransplants graft well, and survive. Many authors are of the opinion that the recipient develops antibodies as an immunity reaction to the homotransplant; but there is some lack of clarity as to whether there is actual formation of antibodies, so the problem has not been settled. Several authors [1, 2] report discovering complement binding antibodies after skin homotransplants in mice and rabbits. Other authors [5, 8], utilizing the complement binding reaction, were unable to find antibodies in dogs after kidney homotransplants, or in rabbits after skin homotransplants. Lately there have been reports of studies with purebred mice. The authors of these studies report [3, 6] that transplanting skin from one of these strains to another produces agglutinins in the recipients against the erythrocytes of the donor, as brought out by the method of determining incomplete, or so-called blocking, monovalent antigens. Similar antigens have been discovered in dogs receiving kidney homotransplants [8]. In the literature there are individual indications on the possibilities of finding antigens after homotransplants with the aid of the usual hemoagglutination reaction.

For example, Curtiss and Herdon [4] discovered in two rabbits, after a homotransplant of a joint, that there were agglutinins to the erythrocytes of the donor. The same authors [5, 7] were unsuccessful in demonstrating antibodies in rabbits (after skin homotransplants) by the aid of the hemoagglutinin reaction.

It seems, therefore, that th : as to antibodies, arising after homotransplants, is a subject for discussion.

EXPERIMENTAL METHOD

For auto- and homotransplants we utilized rabbits of the chinchilla breed weighing 1750-3500 g. The day before surgery both the recipient and donor had the skin of their backs shaved. The day of the operation the operative field was washed with warm, soapy water. Full thickness transplants were done under sterile conditions using ether anesthesia. The skin grafts were separated from the underlying (panniculus carnosus) muscle layer along with the overlying, clearly visible, blood vessel net, shaped into a square or right triangle measuring 60-100 cm^2, and laid into the recipient wound bed in such fashion that the direction of hair growth in the donor patch was opposite to that of the recipient. The transplant was fixed in place with interrupted sutures.

Twenty-three rabbits received homotransplants of the skin while 7 received autotransplants. All the autotransplants lived; observations were continued for half a year and longer. With homotransplants long survival proved unattainable. All the homotransplants died by the end of the first week, or the beginning of the second.

In our investigation we used the hemoagglutination reaction.

We took the serum and erythrocytes from all rabbits before transplantation and frequently thereafter. In our experiments unheated sera were used.

Erythrocytes were thrice washed with physiological saline, after which a standard 2% by weight suspension was prepared. In our work the erythrocytes were never over two days old.

For preparing the reaction, two drops of physiological saline were added to a row of test tubes. To the first tube two drops of serum were added and mixed; two drops transferred to the next tube, etc. In this way serial dilutions were made (from 1:2 to 1:256). Then to each test tube was added a drop of the standard erythrocyte solution. The test tube rack was agitated; test tubes were allowed to stand 2-3 minutes at room temperature and they were then centrifuged at 1500 rpm for one minute. The results were evaluated as to the character of the agglutinate. The serum of each rabbit was studied before and after skin transplantation; the hemoagglutinin reaction was observed in the same rabbit, the erythrocytes of the donor, and also with erythrocytes of other rabbits.

EXPERIMENTAL RESULTS

In Table 1 are shown typical results of the studies of the sera of two rabbits typed with the erythrocytes of 7 other rabbits. From the table it is evident that the serum of rabbit No. 71 and serum of rabbit No. 510 before the skin transplantations contained no agglutinins either against their own erythrocytes or the erythrocytes of other rabbits (46, 866, 756, 407, 442, 173, 786). On the 10th day of an autotransplant of the skin in rabbit No. 71, its serum continued to possess no agglutinins against either its own erythrocytes or those of other rabbits. However, in the same length of time after a homograft of skin the serum of the recipient (Rabbit No. 510) showed agglutinins against the erythrocytes of the donor (Rabbit No. 46), as well as erythrocytes of other rabbits (756, 442, 173). After a homotransplant the serum of the rabbit did not agglutinate its own erythrocytes, or the erythrocytes of rabbits Nos. 407 and 786.

The results of the immunological studies in most rabbits were analogous to those shown in Table 1.

TABLE 1

Typical Protocol of Hemoagglutination Studies Before and After Skin Transplants

Rabbit No.	Serum	Rabbit whose erythrocytes were tested	Serum dilutions 1:2	1:4	1:8	1:16	1:32	1:64	Control autoagglutination (erythrocytes + physiological saline)
71	After autograph of skin	71	—	—	—	—	—	—	—
		46	—	—	—	—	—	—	—
		866	—	—	—	—	—	—	—
		756	—	—	—	—	—	—	—
		407	—	—	—	—	—	—	—
		442	—	—	—	—	—	—	—
		173	—	—	—	—	—	—	—
		786	—	—	—	—	—	—	—
	Before homo transplant of skin	Recipient 510	—	—	—	—	—	—	—
		Donor 46	—	—	—	—	—	—	—
		866	—	—	—	—	—	—	—
		756	—	—	—	—	—	—	—
		407	—	—	—	—	—	—	—
		442	—	—	—	—	—	—	—
		173	—	—	—	—	—	—	—
		786	—	—	—	—	—	—	—
510	After homo transplant of skin	Recipient 510	—	—	—	—	—	—	—
		Donor 46	+—	++	++	++	+	+	—
		866	—	—	—	—	—	—	—
		756	++	++	+(+)	+(+)	+	+	—
		407	—	—	—	—	—	—	—
		442	++	++	++	+	±	—	—
		173	++	++	++	+	±	—	—
		786	—	—	—	—	—	—	—

Before transplantation, we were unable to find autoagglutinins in a single rabbit. After autotransplants all 7 rabbits failed to show isoagglutinins. In contrast to this state of affairs, 15 out of 23 rabbits developed isohemoagglutinins after homotransplants of skin.

The serum of each rabbit developing the hemoagglutinating reaction after a homotransplant was tested against the erythrocytes of 48 other rabbits. Besides the erythrocytes of the donor, it was established that the serum of the recipient agglutinated the sera of some other rabbits, but not the sera of others.

The titer of hemoagglutination among rabbits after homotransplants fluctuates from 1:128 in some rabbits down to only 1:8 in others (Table 2).

TABLE 2

Titer of Hemoagglutinins in Rabbit Sera After Homogenous Skin Grafts

Rabbits	Titer
4	1:128
2	1:64
6	1:32
2	1:16
1	1:8

Not all the rabbits developed antibodies after homogenous skin transplants. The reason for this is not clear. However, it should be observed that of the 8 rabbits with homogenous grafts who did not develop antibodies, in 4 the transplant did not survive (2 developed pus under the transplant, while in 2 rabbits the transplants were torn off by the animal itself on the second postoperative day).

The failure of some workers [5, 7] to obtain hemoagglutinins after homogenous skin transplanting may be due to the insufficient size of the graft. The maximum size of their transplants did not exceed a few cm^2, while our transplants were very much larger.

In the above fashion, we have shown that homogenous skin transplantation leads to the appearance of hemoagglutinins among the rabbit-recipients; these immune bodies, appear to be evidence of the reality of antigenic differences between donor and recipient. When autografts of the skin are done such antigens have not been demonstrated.

The confirmation of the appearance of antigens after homoplastic transplantation enables us to approach the study of the connection between antigen-antibody formation and the dissolution of temporarily surviving homogenous skin transplants.

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