ON THE POSSIBILITY OF FORMING ANTI-ANTIBODIES AND THEIR EFFECT ON THE SURVIVAL OF A SKIN HOMOTRANSPLANT

L. M. Okuneva

(Donetsk Scientific Research Institute of Traumatology and Orthopedics)
(Presented by Acting Member of the USSR Academy of Medical Sciences, N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 57, No. 2, pp. 104-108, February, 1964
Original article submitted July 27, 1962

It is generally recognized that homotransplantation of different tissues is accompanied by an immunological reaction of the organism because of the isoantigenic differences between donor and recipient. As a consequence of this, it is possible to observe antibodies in the blood serum of the recipient and these antibodies are one of the basic causes of the destruction of the transplant [6, 7].

We have used antibody formation as a method for overcoming tissue incompatibility by exploring the antigenic properties of the antibodies. In response to the introduction of antigen (in this particular case antibody) into the tissues there is produced an anti-antibody, which can inhibit the development of tissue immunity and indeed neutralize it. Under such experimental conditions it may be possible to bring about a prolongation of the survival time of skin homotransplants.

As is well known, the amount of antibody found in the blood serum of the recipient after transplantation is small [9, 10, 11] and therefore in order to increase the titer it is necessary to use dense tissue (liver, spleen, lymph nodes). In so doing one takes into account the fact that after homotransplantation a considerable amount of antibody is to be found in the liver, spleen and particularly in the lymph nodes.

EXPERIMENTAL METHOD

Three series of experiments were carried out. In the first series, skin homotransplantation was carried out on 10 rabbits. It was found that the greatest amount of antibody occurred in the blood plasma immediately after sloughing of the homotransplant (confirmed by a hemaglutination titer of 1:128). At this time the liver, spleen, inguinal and suprarenal lymph nodes were removed from one animal and an emulsion prepared from

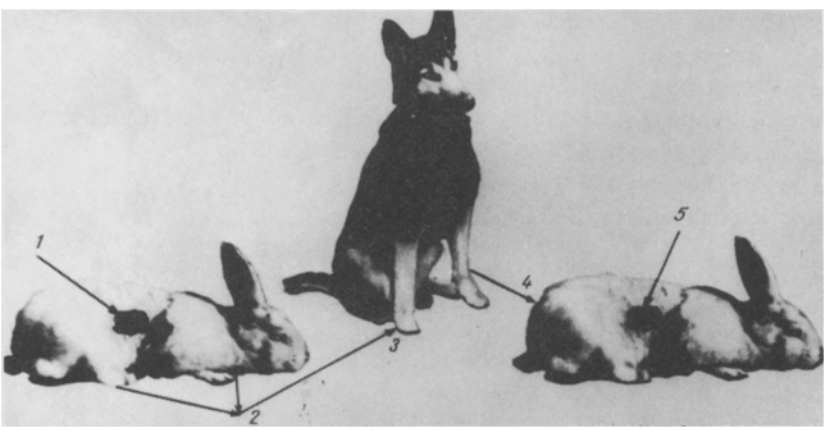

Diagram of experiment. 1) Transplant in rabbit, first series of experiments; 2) removal of lymph nodes from rabbit, first series of experiments; 3) emulsion of lymph nodes used to immunize dog; 4) blood plasma of dog used to immunize rabbit, second series of experiments, 5) transplant in rabbit of second experimental series.

Determination of the Amount of Protein in the Blood Plasma of Experimental Rabbits

No. of rabbit	Before immunization					After immunization			
	total protein	albumen	globulin	protein coefficient	antigen	total protein	albumen	globulin	protein coefficient
51	5,25	2,97	2,28	1,3	Liver emulsion	7,42	2,93	4,49	0,65
52	6,55	3,82	2,73	1,43		7,85	3,61	4,24	0,84
53	7,20	3,29	3,91	0,84		7,20	2,70	4,50	0,60
54	6,87	3,23	3,64	0,88		7,20	3,20	4,00	0,80
55	6,50	3,20	3,30	0,97		7,22	2,90	4,32	0,67
56	7,20	3,42	3,78	0,90		7,20	2,70	4,50	0,60
Means . . .	6,59	3,32	3,27	1,06		7,34	3,0	4,34	0,69
61	6,50	3,80	2,70	1,40	spleen emulsion	7,20	2,06	5,14	0,40
62	6,55	3,78	2,77	1,36		7,85	3,61	4,24	0,85
63	6,55	3,90	2,65	1,47		6,55	3,58	2,97	1,20
64	7,42	3,26	3,16	1,03		7,20	2,70	4,50	0,60
65	7,20	2,88	4,32	0,66		8,28	2,94	5,34	0,55
66	6,80	3,20	3,60	0,88		7,30	2,16	5,14	0,42
Means . . .	6,83	3,65	3,6	1,13		7,39	2,84	4,55	0,67
71	7,10	3,32	3,78	0,87	lymp node emulsion	7,40	2,26	5,14	0,43
72	7,40	3,97	3,43	1,15		8,06	3,72	4,34	0,83
73	7,40	3,49	3,91	0,89		7,20	2,56	4,64	0,55
74	7,50	3,75	3,75	1,00		7,20	2,70	4,50	0,80
75	6,50	3,90	2,60	1,50		8,16	3,72	4,44	0,83
76	7,55	3,82	3,73	1,02		8,30	2,96	5,34	0,53
Means . .	7,24	3,70	3,46	1,24		7,72	2,98	4,72	0,62
81	7,30	3,70	3,60	1,02	Emulsion from organs of intact rabbit	7,50	3,50	4,20	0,87
82	6,80	3,50	3,30	1,06		7,35	3,50	3,85	0,90
83	6,90	3,45	3,45	1,00		7,45	3,45	4,00	0,87
84	7,10	3,70	3,40	1,08		8,00	3,70	4,30	0,86
85	6,80	3,20	3,60	0,90		7,30	2,16	5,14	0,42
86	7,00	3,20	3,80	0,80		7,40	3,40	4,00	0,85
Means . . .	6,98	3,45	3,52	0,97		7,50	3,28	4,24	0,78

them. In order to prepare the emulsion 1 g. of tissue was ground in a mortar with 4 ml. of physiological saline and the resultant mixture was filtered through gauze to free it from suspended particles. The filtrate was diluted with 5 times its volume of physiological saline and used to immunize the dog.

In the second series of experiments, an emulsion of rabbit organ material from animals of the first experimental series was used to immunize 3 dogs according to the following scheme: 0.5 ml of liquid was injected twice subcutaneously and five times intravenously. Intervals between injections were of 3-4 days duration. It should be understood that the rabbit organ emulsion contained transplantational antibody (which acted as an antigen toward the dog). As a result of the injection of this material into the dog there appeared specific antibodies (subsequently referred to as first order antibodies).

Blood plasma from each of the dogs was injected into 16 rabbits. In the blood of these animals there appeared anti-antibodies of the second order. The intensity of immunity in these animals was determined by measuring the increase in the globulin fraction of plasma protein [13].

Later the immunized rabbits were given skin homotransplants taken from the animals which had served as the original donors.

The third control series of experiments differed only in that the three dogs were injected with an emulsion prepared from organs of rabbits which had not received homotransplants. The blood serum from these animals was used to immunize 2 rabbits. Transplantation of the skin graft was carried out in all cases using the method described by E. A. Zotikov [9].

Sixty-five chinchilla rabbits of mean weight from 1600 to 2200 g were used in this research; in addition we used 6 dogs. The general scheme of the experiments is shown in the figure.

In the first series of experiments, involving 10 non-immunized rabbits, the transplanted portions of skin were initially accepted. However, between the 7th and 10th day they all sloughed off. The blood plasma of these animals

was found to have a low titer (1 : 32), as confirmed by the hemaglutination reaction. Injection of plasma from dogs immunized against rabbit organ emulsion invoked a change in the blood protein fraction of the recipient rabbits (second series of experiments). This involved an increase in the globulin fraction as indicated by a change in the protein coefficient (Table 1).

The greatest increase in globulin is associated with immunization using lymph node emulsion, the next greatest with spleen emulsion and after that liver emulsion.

Increase in the amount of globulin in blood plasma is usually related to the formation of antibodies, among which we may include second order anti-antibodies formed in these experiments.

Transplants made on rabbits of the second experimental series survived until the 6th day. Their sloughing took place considerably later than that of animals belonging to the first experimental series (Table 2). Thus, after injection of liver emulsion sloughing was completed in 15-24 days after transplantation (a considerable portion of the transplant sloughed off during the 20-24 day period).

In rabbits immunized with plasma from dogs given injections of spleen emulsion the survival time of the transplant was somewhat greater (sloughing accomplished in a mean time of 20-25 days).

The longest period of time that any transplant survived was on those occasions when plasma from dogs immunized with lymph node emulsion was used. In these cases sloughing of the main mass of transplant did not occur until the 24-32 day period (as a rule, it commenced on the 30th day after transplantation).

The results of the control series of experiments indicated that some increase in the globulin fraction of rabbit blood does take place without any corresponding increase in the survival time of the skin transplant. In these cases sloughing commenced on the 7-11th day after transplantation, i.e., at the same time as in the first series of experiments. It would appear that formation of the antibody occurred in the animals in response to the presence of foreign protein but that anti-antibodies did not occur.

On the basis of what has been described above we conclude that the increase in survival time of skin transplants in the second series of experiments is explainable in terms of the effect of anti-antibodies of the second order.

TABLE 2. Period of Survival of Skin Homotransplants in Experimental Rabbits

Immunization emulsion					
liver		spleen		lymph nodes	
No. of cases	day when sloughing of transplant was accomp.	No. of cases	day when sloughing of transplant was accomp.	No. of cases	day when sloughing of transplant was accomp.
1	15	1	9	3	24-31
2	18-	1	18	1	25
2	19-	3	20-	2	27
6	20-	1	21-	2	28
4	21	4	22	4	30-
1	24	2	23	3	31
		1	24	1	32
		3	25-		

SUMMARY

A study was made of the effect produced by anti-antibodies on the survival period of skin homotransplants. Rabbits and dogs were used to obtain the anti-antibodies.

In homotransplantation of the skin (in rabbit) the transplant sloughs off on the 7th-10th day. Rabbit immunization with a heterogeneous protein(blood serum of dog, immunized with an emulsion of organs of rabbits not subjected to skin transplantation) does not affect the period of take of the skin homotransplant. Immunization of rabbits with an antigenic complex, the composition of which consists of tissue proteins and anti-antibodies of the first order, increases the period of take of the transplanted skin graft by 3-4 times as compared to control.

An increase of the period of take of skin homotransplants of rabbits is connected with the action of anti-antibodies of the second order.

The liver, spleen and lymph nodes are of great significance not only in the appearance of the transplantation immunity, but they also may play an important role in overcoming it.

LITERATURE CITED

1. G. A. Annenkov, Collected Works from the Kursk Medical Institute, 2, p. 335 (1956) [in Russian].
2. S. D. Balakhovskii and I. S. Balakhovskii, Methods for the Chemical Analysis of Blood, Moscow (1953) [in Russian].
3. Ya. Brubel', Eksper. zhir. (1962), 1, p. 50.
4. L. I. Geller, Zh. Mikrobiol., 10, 73 (1958).
5. N. N. Dakhina, Proceedings of the 2nd All-Union Conference on Problems of Tissue Incompatibility, Conservation and Transplantation of Tissues and Organs "Odessa," p. 23 (1961) [in Russian].
6. N. N. Zhukov-Verezhnikov, P. M. Chepov, M. M. Kapichnikov, et al., Eksper. zhir. (1957), 2, p. 55.
7. N. N. Zhukov-Verezhnikov, Problems of Transplantation and Conservation of Organs and Tissues, Moscow (1959), [in Russian].
8. L. A. Zil'ber, Vestn. AMN SSSR, 10, p. 60 (1959).
9. E. A. Zotikov, Byull. éksper. biol., 7, 58 (1956).
10. E. A. Zotikov, Byull. éksper. biol., 12, 91 (1957).
11. P. M. Medvedev and L. S. Priezzheva, Problems of Transplantation and Conservation of Organs and Tissues, Moscow (1959) [in Russian].
12. A. I. Polyak, Antibody formation in lymphoid tissue and the influence of ionizing radiation on this process, Candidate's Dissertation, Rostov-na-Dony (1961).
13. V. E. Predtechenskii, V. M. Borovskaya, and L. T. Margolina, Manual of Laboratory Research Methods, Moscow (1950), [in Russian].
