THE DETECTION OF HEMAGGLUTININS IN REGIONAL LYMPH NODES FOLLOWING SKIN HOMOTRANSPLANTATION IN RABBITS

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It has been established that the cause of tissue incompatability following homotransplantation is the antigenic difference between the donor and the recipient [2, 5]. However opinions on the mechanism of the immunologic response of the recipient to the homotransplant differ. Some authors favor the significance of cellular factors [9, 10, 13-17] and other humoral factors [5, 11, 12].

The original data on the participation of lymphoid tissue in antibody formation were reported at the end of the last century [3, 4]. In one of these investigations [7] it was shown that after immunization of dogs by the antigen of paratyphi B the antibody titers are higher in extracts of regional lymph nodes than in the blood, lymph or distant nodes. The work of other authors [1, 8] demonstrated the relation between immunologic response and the plasma cells of the organism. Studies of the cellular reactions in regional lymph nodes after transplantation of normal tissue are few in number [15, 16]. Workers studying morphologic changes in regional lymphatics and in the spleen following skin homotransplant to rabbit ears noted that cytologic changes first occur in regional nodes, especially in the cortex. This reaction corresponds to the active phase of immunity and consists of the formation of "large lymphoid cells" [15]. Other workers call them "plasma cells of the transitional type," "lymphoblasts," etc. These same investigators noted that in the spleen and other lymph nodes following skin homotransplantation little cellular reaction is noted. They hypothesize that "large lymphoid cells" are concerned with antibody formation. After skin homogransplantation in the rabbit hemagglutinins are not always found in the blood.

The present study is an attempt to demonstrate antibodies in the cells of the regional lymph nodes following skin homotransplantation in the rabbit.

EXPERIMENTAL METHODS

Rabbits received homotransplants of skin measuring 2 x 3 cm taken from the ear of the donor rabbit. Autotransplanted skin of the same dimensions was placed on the opposite ear simultaneously as a control.

Before the homotransplantation of the skin hemagglutinins to the erythrocytes of the future donor could not be detected in the recipients.

For detection of the hemagglutinins in the cells of the regional lymph nodes the parotid lymph node situated along the course of the ear vein at the base of the ear was separated. The hemagglutinin content of the serum of the recipient rabbit was measured prior to this. The operations were performed on the 6th, 7th, 8th, 9th, 11th or 17th days following the skin transplant on recipient rabbits in whose sera no hemagglutinins could be found.

The liberated nodes were washed in saline, weighed, and imprints were made on glass slides by the method of M. P. Pokrovskaya [6]. The imprints were fixed with methyl alcohol and stained with Romanovsky-Giemsa stain.

A saline extract of the nodes was prepared for the determination of antibody. The node tissue was suspended in 1 ml of saline per 100 mg of node tissue. The supernatent fluid following centrifugation (extract) served as the material for determination of hemagglutinins to erythrocytes of the donor.

Thirty-six rabbits in whose sera no hemagglutinin was found following skin homotransplant to the ear were investigated.

Investigation of the extracts from the regional nodes on the experimental side revealed the presence of hemagglutinins in 19 rabbits. From the table it is evident that antibody in low titer – from 1:2 to 1:8 was present. In 9 rabbits of the 19 hemagglutinins were found in the regional nodes from the control side. These contained even less antibody, in a titer of 1:2 or 1:4.

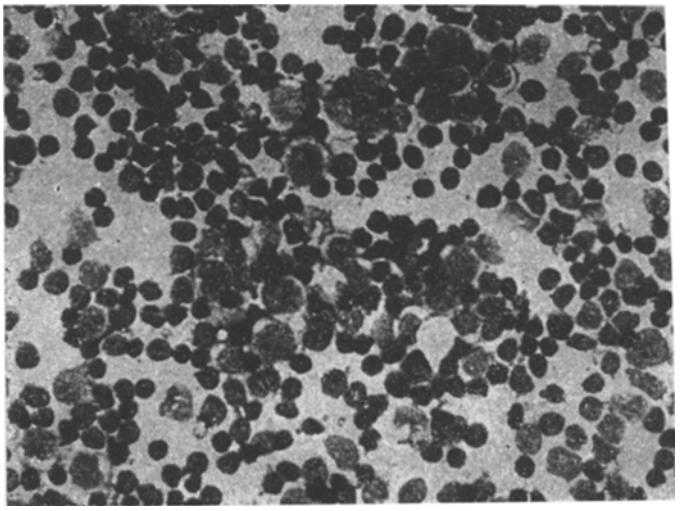

Fig. 1. Node imprint from rabbit No. 115 seven days after skin homotransplant. Many "large lymphoid cells" are seen. Magnification 280.

The Hemagglutination Reaction to Donor Erythrocytes from Extracts of Recipient Nodes

Rabbit No.		Post-operative day	Experimental				Control			
			dilution of extract							
recipient	donor		undiluted	1:2	1:4	1:8	undiluted	1:2	1:4	1:8
230	253	6th	+	+	+	+	±	±	–	–
257	267	"	+(+)	+(+)	+(+)	+	+	+	+	–
36	60	"	–	–	–	–	–	–	–	–
100	109	7th	+	+	±	–	–	–	–	–
28	112	"	–	–	–	–	–	–	–	–
115	109	"	+	+	+	–	–	–	–	–
b/No.	856	8th	+	±	–	–	–	–	–	–
778	771	"	+	±	–	–	–	–	–	–
795	774	9th	+++	+	–	–	–	–	–	–
824	774	"	+++	+	±	–	–	–	–	–
706	774	"	–	–	–	–	–	–	–	–
822	774	"	+	+	–	–	–	–	–	–
82	10	11th	++(+)	++	++	+	++	+(+)	+	–
52	51	"	++	+(+)	±	–	++	+	–	–
54	9	"	–	–	–	–	–	–	–	–

In studying the morphologic picture of the imprints it was noted that the experimental nodes gave a denser more cellular preparation than the controls.

Many "large lymphoid cells" can be seen in the imprints (Fig. 1). These cells are round with a large nucleus which occupies almost the entire cell. The cytoplasm is basophilic surrounding the nucleus as a wreath. Frequently somewhat smaller cells are encountered with a deep blue cytoplasm.

In the control imprints (Fig. 2) such a cellular reaction was not noted in any of the animals. "Large lymphoid cells" were rarely encountered. Mature plasma in the experimental and the control was isolated. The cellular

reaction in the lymph nodes resembled the active phase of immunity. It is considered that in the elaboration of antibody plasma cells are the final stage of the conversion of lymphoid and reticular cells. In agreement with other data antibody is elaborated by the young forms of plasma cells [3]. These young forms – "large lymphoid cells" – are evident in great numbers in the experimental lymph nodes. We found that in all the rabbits the weight of the lymph nodes on the experimental side was 2 to 3 fold greater than on the control side. Thus it was established that antibody formation in regional lymph node cells following skin homotransplantation occurred even in the absence of serum antibody. The absence of antibody from the serum apparently can be explained by the fact that the regional lymph nodes elaborate small titers of antibody which upon entrance into the general circulation are so diluted that they cannot be detected.

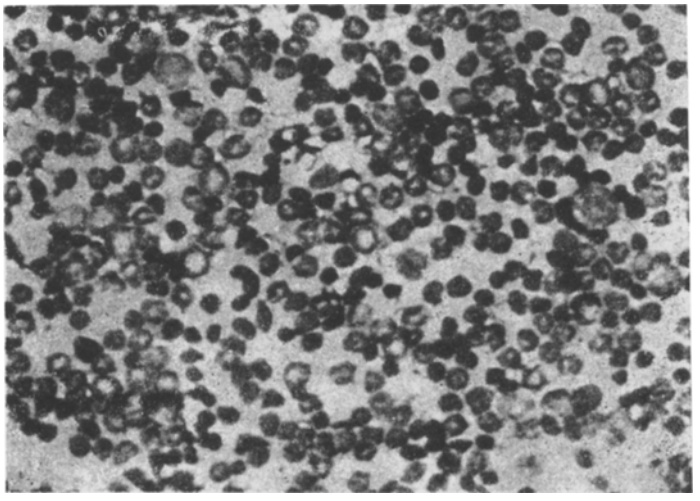

Fig. 2. Node imprint from rabbit No. 115 seven days after skin autotransplant (control). "Large lymphoid cells" are rarely encountered. Magnification 280.

SUMMARY

Hemagglutinins were investigated in extracts of rabbit regional lymph nodes following homotransplantation of skin to the ears. The regional lymph node of the other ear of the same animal after simultaneous autotransplantation of the skin served as the control.

In 19 of 36 rabbits antibodies were revealed in lymph node extracts; these antibodies were not present in the serum. The antibodies were detected in 9 controls, but their titre was lower than in the experimental animals. Thus, the author confirmed that antibodies are formed in the cells of rabbit regional lymph nodes in response to homotransplantation of skin.

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