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PROLONGATION OF RABBIT-TO-DOG LIVER XENOGRAFT SURVIVAL IN RECIPIENTS PRETREATED WITH RABBIT BLOOD

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An attempt was made to prolong rabbit-to-dog liver graft survival by pretreatment of recipients with donor species blood elements. Recipients were given intraarterially whole blood, washed erythrocytes, erythrocyte stroma, leukocytes, and rabbit plasma 1 hr befor transplantation of rabbit liver.
Pretreatment with whole blood or red cells was followed by acute depletion of hemagglutinins, hemolysins, complement, and decrease in platelet and leukocyte counts. Rabbit livers transplanted at that time had a 10—20-fold longer survival time. Pretreatment with donor leukocytes only slightly prolonged graft survival, whereas rabbit plasma had no effect at all. It may be concluded that recipient circulating antibodies agglutinating donor erythrocytes are responsible for triggering the process of hyperacute rejection.

In previous publications (Olszewski et al., 1972, 1973c) we described the immunological phenomena in rabbit liver transplanted to the dog. The purpose of those studies was to observe the process of hyperacute rejection of a graft transplanted between highly discordant species and to compare the pathophysiological and immunological data obtained during an animal experiment with those from the porcine liver perfusion with human blood (Olszewski et al., 1973a). The data revealed a close similarity of the immunological phenomena in both interspecies transplantations, i. e. rabbit-to-dog, and pig-to-man, indicating that the same biological factors are responsible for graft function deterioration and its subsequent destruction. It was found that recipient serum heteroagglutinins to donor erythrocyte and leukocyte antigens, as well as complement, were avidly consumed by the graft. This phenomenon together with platelet and leukocyte trapping correlated in time with the deterioration of the graft perfusion and function. It may be infered from these findings that natural hemagglutinins and leukoagglutinins initiate the intravascular immune reaction and are responsible for the hyperacute rejection of xenografts.

The purpose of the present study was to investigate whether the in vivo depletion of the recipient of the circulating heteroantibodies by pretreatment with donor blood elements results in prolongation of graft survival.

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MATERIAL AND METHODS

Eighteen rabbit-to-dog liver transplantations were performed in 5 groups (Table 1). In group 1, 60 min before transplantation recipients were given intraarterially 20 ml/kg of body weight of rabbit whole blood. Rabbit blood was injected intraarterially to avoid trapping of cells in the lungs and thus to attenuate the anaphylactic reaction. In group 2, dogs received intraarterially 10 ml/kg of body weight of rabbit packed red cells. In

Table 1. Pretreatment of recipient dogs with donor rabbit antigen (blood elements) before rabbit liver transplantation

Group	Number of dogs	Pretreatment	Time of rabbit liver transplantation
1	3	Rabbit whole blood 20 ml/kg, intraarterially	60 min
2	3	Rabbit erythrocytes, 3× washed with saline 10 ml/kg of packed cell mass, intraarterially	60 min
3	6	Rabbit erythrocyte stroma prepared from 10 ml/kg od erythrocytes, intraarterially	60 min
4	3	Rabbit leukocytes from buffy coat, prepared from 20 ml/kg of whole blood, intraarterially	60 min
5	3	Rabbit platelet-poor plasma, 10 ml/kg, intraarterially	60 min

group 3, rabbit erythrocytes were hemolyzed with distilled water, centrifuged, and stroma in an equivalent of 10 ml/kg body weight of packed red cells was injected intraarterially. In group 4, the buffy coat was skimmed from rabbit whole blood and leukocytes obtained from 20 ml/kg body weight of blood were infused. In group 5, 10 ml/kg body weight of platelet-poor plasma was injected intraarterially. One hour later, rabbit liver was transplanted to the dog by the technique described previously (Olszewski, 1973b). Graft blood flow was measured every 5 minutes by collecting the effluent in a calibrated cylinder and expressed in ml/g/min. Rejection of the graft was judged by cessation of blood flow and change in its gross appearence. Blood samples were taken for immunological studies from the peripheral vein before administration of rabbit blood elements and 60 min thereafter, then from the in and outflow line 5, 30, 60 and 120 min after transplantation. The following parameters were measured: dog serum hemagglutinin, hemolysin, leukoagglutinin, lymphocytotoxin titers to rabbit erythrocyte or lymphocyte antigens, total complement, platelet and leukocyte count.

Table 2. Changes in dog blood 60 min after intraarterial pretreatment with rabbit blood elements

	Hemagglutinins	Hemolysins	Leuko-agglutinins	Lympho-cytotoxins	Platelets	Leukocytes	C'
Whole blood	↓	Hemolysins	↓	?	↓	↓	↓
Erythrocytes	↓	Hemolisins	0	?	↓	↓	↓
Erythrocyte ghosts	↓	↓	0	?	↓	↓	↓
WBC	0	0	0/↓	?	↓	↓	0
Plasma	0	0	0	?	0	↓	0

Table 3. Cumulative data of immunological changes in recipients following pretreatment with donor-species blood elements, and graft survival (rejection judged by flow below 0·05 ml/g/min)

Group	Pretreatment	Immunological factors in recipients serum at the time of transplantation						Survival time in minutes
		Hemagglutinins	Hemolysins	Lympho-agglutinins	C'	Platelets (mean values)	Leukocytes (mean values)	
Control	No.	1:4	1:16	1:2—1:4	17·6	293 000	10 041	
1	Whole blood	0	Hemolysis	0	6·5	182 000	4 800	60, > 120, > 120
2	Erythrocytes	0	Hemolysis	1:4	4	115 000	3 900	60, 120, > 120
3	Stroma	0	0—1:8	1:4	5·3	105 000	3 300	20, 60, 60, 60, 120, 120
4	White blood cells	1:2—1:4	1:8	0—1:2	12·1	204 000	3 300	30, 30, 120
5	Plasma	1:4	1:8—1:16	0—1:4	22·7	150 000	6 400	5, 7, 8

RESULTS

Pretreatment. Immunological changes in dog blood after pretreatment with rabbit blood elements are shown in Table 2. Titers of hemagglutinins decreased after administration of whole blood, erythrocytes and stroma to zero. Leukocytes and plasma did not affect hemagglutinin and hemolysin titers. Lymphoagglutinin titers were slightly diminished after leukocyte infusion. Lymphocytotoxic titers did not change or increased slightly, the reason for which is not clear and needsfurther study. Platelet counts decreased considerably after administration of rabbit cells, but did no change after injection of rabbit plasma. Leukocyte counts diminished in all the groups. Total complement level was affected only by whole blood, erythrocytes, or stroma pretreatment.

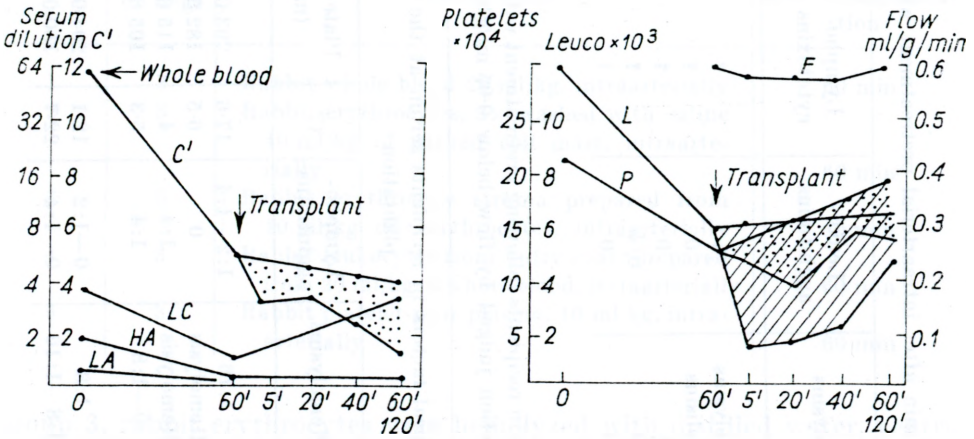

Fig. 1. Rabbit-to-dog liver transplantation in a recipient pretreated with 20 ml/kg body weight of rabbit whole blood. HA — hemagglutinins, HL — hemolysins, LA — lymphoagglutinins, LC — lymphocytotoxins, C' — total complement, F — blood flow, L — leukocytes, P — platelets. Shaded areas-gradient across the graft.

Transplant survival and immunological changes

The cumulative data are presented in Table 3.

Group 1. There was a prolongation of graft survival in 3 consecutive experiments, from the control 7 min to 60, >120, and >120 min (Fig. 1). No changes in the external appearence of the liver were observed until 40—60 min. In one dog totally depleted of C', graft blood flow remained at a constant level for 120 min of perfusion. In all cases slight consumption of antibodies and retention of cells was noted within the graft.

Groupe 2. Graft survival was prolonged in 3 experiments to 60, 120, and 120 min. There was a steady decrease in blood flow in two experiments (Fig. 2). In the third recipient, another rabbit liver grafted on the next

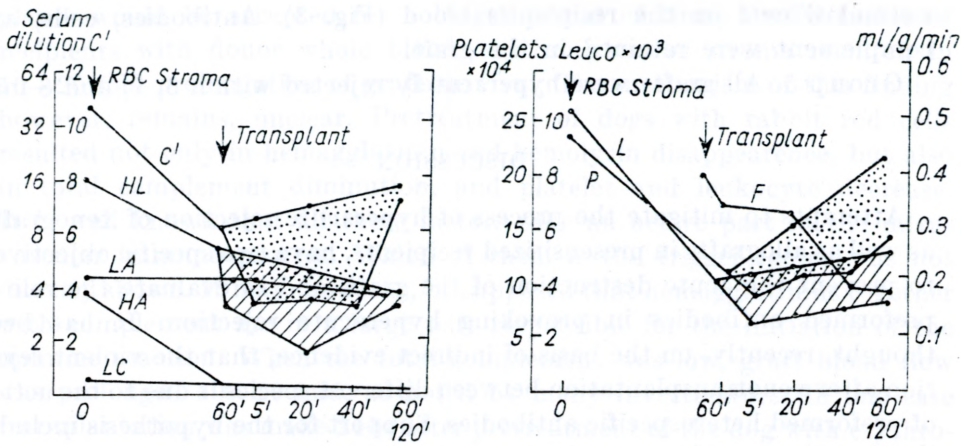

Fig. 2. Rabbit-to-dog liver transplantation in a recipient pretreated with rabbit erythrocyte stroma (equivalent to 10 ml/kg of packed erythrocytes). For details see Fig. 1.

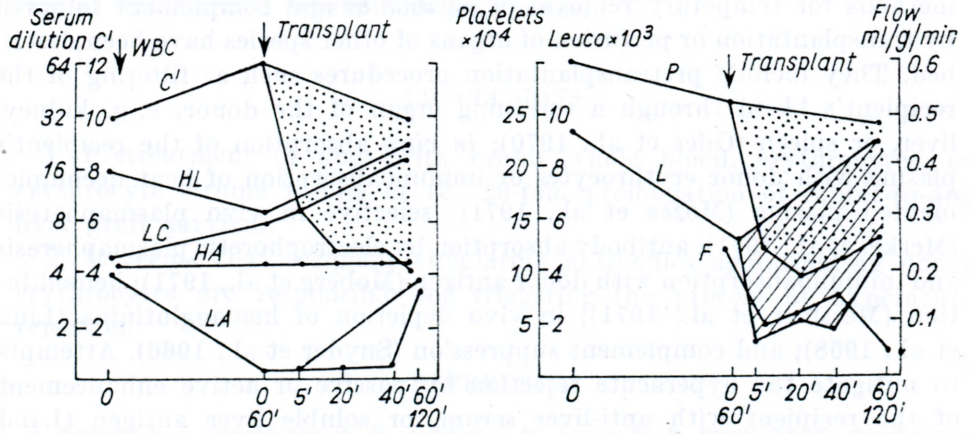

Fig. 3. Rabbit-to-dog liver transplantation in a recipient preated with rabbit white bloo d cells (buffy coat of 20 ml/kg blood). For details see Fig. 1.

day without pretreatment with rabbit erythrocytes survived uneventfully for 120 min. The third transplant given the same dog 7 days later was rejected within 3 min. In all the transplants, retention of antibodies, complement and cells was observed within the graft.

Group 3. Grafts survived 20, 60, 60, 60, 120, and 120 min. On the whole, flows were lower than in the two previous groups. Livers became bluish and mottled already after 10—15 min. There was an evident gradient of antibodies, complement, and cells across the transplant.

Group 4. Two livers survived 30 min, and one almost two hours despite normal C'level on the recipient's blood (Fig. 3). Antibodies, cells, and complement were retained in the graft.

Group 5. All grafts were hyperacutely rejected within 5, 7, and 8 min.

DISCUSSION

Attempts to mitigate the process of hyperacute rejection of xenografts, as well as allografts in presensitized recipients, have two specific objectives: to prevent the acute destruction of the graft, and to evaluate the role of preformed antibodies in provoking hyperacute rejection. It has been thought, recently, on the basis of indirect evidence, that the violent rejection after xenotransplantation between different species is due to the action of preformed heterospecific antibodies. Support for the hypothesis includes the fact that antidonor antibodies were demonstrated often by pre-transplantation in vitro testing of the recipient's serum (Perper et al., 1966), and also that attempts to deplete the recipient of circulating antibodies resulted in prolongation of graft survival. Numerous *in vivo* and *in vitro* methods for temporary removal of antibodies and complement followed by transplantation or perfusion of organs of other species have been described. They include pretransplantation procedures such as filtering of the recipient's blood through a screening organ of the donor, e. g. kidney, liver, or spleen (Giles et al., 1970); *in vitro* absorption of the recipient's plasma with donor erythrocytes or immunoabsorption of heat decomplemented plasma (Mozes et al., 1971); selective in vivo plasmapheresis (Merkel et al., 1971); antibody absorption by electrophoretic plasmapheresis and immunoabsorption with donor antigen (Moberg et al., 1971); hemodilution (Messmer et al., 1971); in vivo depletion of hemagglutinins (Linn et al., 1968); and complement suppression (Snyder et al., 1966). Attempts to mitigate the hyperacute rejection by passive or active enhancement of the recipient with anti-liver serum, or soluble liver antigen (Land et al., 1972), and by leukocyte depletion (Meija-Laguna et al., 1971) should also to mentioned. None of the above mentioned studies, however, have revealed the existence of a specific antibody responsible for hyperacute rejection. Only depletion of the recipient blood of all gamma-globulins, that is all circulating antibodies, and /or total complement prolonged graft survival. It seems, however, that in transplantation between different species hemagglutinins and hemolysins as the oldest natural antibodies

probably play a role in the hyperacute rejection. Their titers are usually much higher than those of all the other heteroantibodies. In our study, therefore we tried to deplete the recipient temporarily of hemagglutinins and hemolysins to donor red cells, in order to ascertain whether the transplants would be accepted for a longer period of time, Pretreatment of recipients with donor whole blood, erythrocytes, or stroma resulted in 10—20 fold prolongation of graft survival. The mechanism of this finding however, remains, unclear. Pretreatment of dogs with rabbit red cells resulted not only in hemagglutinin and hemolysin disappearence, but also in total complement diminution, and platelet and leukocyte decrease. It is well known that all these factors take an active part in the intravascular immune process. Which of them is the trigger factor was not directly shown. It may, however, be supposed that hemagglutinins together with complement are in a large part responsible for the initiation of the immune reaction. When the total complement was low, graft blood flow was prolonged 10—20-fold, as noted by many investigators. In one case transplantation was made 24 hr after pretreatment of the dog with erythrocytes when the hemagglutinin titer was zero, but complement level had returned to normal values. In this case the transplant survived uneventfully for 120 min. The latter observation seems to support the concept that the heterohemagglutinins trigger the rejection process during transplantation of vascularized organs between species.

CONCLUSIONS

1. Pretreatment of dogs with rabbit whole blood, erythrocytes, or erythrocyte stroma results in 10—20-fold prolongation of rabbit-to-dog liver graft survival.

2. It seems that recipient circulating antibodies agglutinating donor erythrocytes are responsible for triggering the process of hyperacute rejection.

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