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RABBIT-TO-DOG LIVER XENOGRAFT AS A MODEL FOR STUDY OF THE PROCESS OF HYPERACUTE REJECTION

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An experimental model of rabbit-to-dog liver transplantation has been designed for studies of the process of hyperacute rejection. Technical details of the transplantation have been presented, as well as the serological differences between the normal subjects of the two species.

Marked immunological disturbances observed in patients with liver insufficiency treated with extracorporeal pig liver perfusion prompted us to study this clinically important problem in animals. Various genetically discordant systems have been proposed in the literature (Rosenberg et al., 1969; Giles et al., 1970; Hammer, 1972; Messmer et al., 1971) for studies of hyperacute rejection of organ transplants between species. Among them, the following have been used in most laboratories: pig-to-dog, sheep-to-dog, and rabbit-to-dog. Kidney, liver, or any other vascularized organ transplanted from the pig, sheep, or rat to the dog are rejected within minutes after reestablishment of blood flow. The first major symptom of rejection is the rapidly increasing vascular resistance of the organ and decrease in blood flow. The hemodynamic changes seem to be directly dependent on intravascular immunological phenomena which occur immediately after the recipient blood comes into direct contact with the endothelial lining of the transplanted organ vasculature. The basic immunological differences between the representatives of donor and recipient species should be thoroughly investigated before experiments on inter-species transplantation can be carried out. Technical factors such as size of the organ, diameter of vessels and sensitivity of the organ to ischemia, should also be taken into account. The purpose of the present study was to investigate the humoral immunological differences between the rabbit and dog, as the donor and recipient of the prospective liver graft. Technical details of liver transplantation were also elaborated.

MATERIAL AND METHODS

1. Technique of liver transplantation (Fig. 1)

Preparation of the recipient. Mongrel dogs of average weight 12 kg were used for the experiments. Under Eunarcon 0·4 ml/kg body weight anesthesia, longitudinal incision on the lateral part of the neck was made, and the superficial jugular vein and carotid artery were exposed. The artery was prepared for anastomosis by grafts with the hepatic artery, whereas the distal part of the jugular vein served as source of blood for the portal vein. The outflowing blood was directed to the proximal segment of the jugular vein.

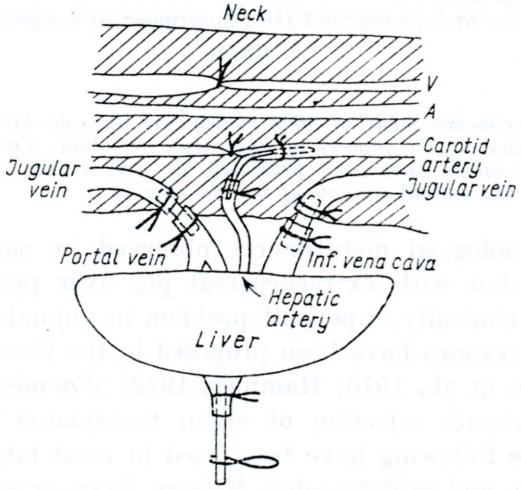

Fig. 1. Technical details of rabbit-to-dog liver transplantation.

Preparation of the donor. Rabbits of average weight 3·5 kg were used for the experiments. Pretreatment consisted of an i. v. injection of 5 mg/kg body weight of heparin sodium, then 5 mg/kg of Dibenzyline (Smith and Kline), to prevent intravascular clotting and vasoconstriction during the liver's removal. Five minutes later, animals were sacrificed with an i. v. bolus of 10 ml of 10% potassium chloride. A long longitudinal incision from the neck to the symphysis pubis was made and the abdominal cavity and thorax were opened. The superior mesenteric vein was cannulated, and the liver was washed out with 1000 ml of Ringer's solution of 25°C. With this amount of solution practically all the blood could be removed from the graft so that only single erythrocytes were found under the microscope at the end of the washing. This is of prime importance as even

the smallest amount of donor blood left in the organ could initiate the immunological reaction after transplantation. The liver was removed using the same technique that is routine in dog-to-dog transplantation (Olszewski et al., 1973).

One end of the Scribner's fistula was introduced into the hepatic artery of the graft. The portal vein, supra-, and infrahepatic IVC were cannulated with 3 cm long silastic tubings. They served later for anastomosis with recipient blood vessels.

Transplantation of the liver. Rabbit liver was transplanted to the dog neck, intrahepatic IVC cannula introduced into the proximal jugular vein of the dog, portal vein cannula into the distal jugular vein, and Scribner's shunt into the carotid artery. The suprahepatic IVC cannula served for blood sampling and flow measurements. Vascular clamps were removed, gross appearence of the liver carefully watched, and blood flow measured every other minute. The recipient dog was heparinized 1—5 mg/kg body weight, to prevent clotting in the anastomosing cannulas, with the exception of experiments in which blood coagulation and fibrinolysis studies were carried out.

2. Immunological studies

These included measurement in dog sera of titers of preformed circulating antibodies to rabbit erythrocyte and lymphocyte antigens, precipitating antibodies to rabbit serum proteins, and total complement activity.

Hemagglutinin and hemolysin titers were measured by the technique described by Ślopek (1970), complement activity by the method of Kabat et al., (1961), lymphocytotoxins and lymphoagglutinins by histocompatibility testing conf. (Nat. Acad, Sci. Washington 1965), and serum precipitating antibodies according to Ślopek, (1970).

RESULTS

1. Technical studies

Twenty rabbit livers prepared by the method described above were anastomosed with dog neck blood vessels. The graft filled within 1 min with blood, its external appearence initially resembling that of an allograft. After a period of about 3—4 min, the graft surface became dark-red and mottled, changing to darkbrown at 7 min. Concurrently with the change in gross appearence, blood flow decreased. Physiologically, liver blood flow should be 0·5—1·0 ml/g/min. In liver xenografts it ranged initially

between 0·15 and 0·5, decreasing below 0·05 ml/g/min at 7 minutes. The drop in blood flow below 0·05 was considered to be consistent with rejection.

2. Immunological studies (Table 1)

Dog-to-rabbit erythrocyte preformed serum hemagglutinins ranged between 1:2 to 1:32, in most cases 1:4. Dog-to-rabbit erythrocyte hemolysins were 1:16 in all the animals. Dog-to-rabbit lymphocyte serum aggluti-

Table 1. Preformed circulating antibodies in dog serum to rabbit erythrocytes and lymphocytes

Dog serum / Rabbit cells	Hemagglutinins	Hemolysins	Lymphoagglutinins	Lymphocytotoxins
Erythrocytes	1:4 (1:2—1:32)	1:16 (1:8—1:16)	—	—
Lymphocytes	—	—	1:4 (1:2—1:16)	14% (0—29%) dead cells in dilution 1:2

nins ranged between 1:2 and 1:16, on the average 1:4. Dog-to-rabbit lymphocyte serum cytotoxins were 1:2 in all the tested dogs. It was decided to use the number of dead lymphocytes in dilution 1:2 as an index of cytotoxicity. The number of dead cells in dilution 1:2 ranged from 0 to 29, on the average 14. No antibodies to rabbit serum proteins were found in dog serum.

DISCUSSION

Rabbit-to-dog liver gafting has proved to be a satisfactory model for immunological studies on the process of hyperacute rejection. The time of rejection was short, not exceeding 10 minutes. Technically, the procedure of transplantation was simple. Collection of affluent and effluent blood was possible, as well as flow measurements and angiography. The titers of preformed circulating antibodies to rabbit RBC, representatives of phylogenetically oldest anti-species antibodies, and to rabbit lymphocytes, were high enough to serve as indicators of the immunological events

developing in the transplant. Species of great genetic divergency were chosen purposely to obtain a rapid and quantitatively measurable immunological and clinical response. The average titers of natural hemagglutinins in carnivorous sera to rodent RBC has been estimated at 1:16 and of leucoagglutinins to rodent lymphocytes at 1:8 (Hammer, 1972). The Forssman antigen is not responsible for the rejection reaction as rabbits do not have it, whereas dogs posess the antigen in most organ cells (Tanaka Nobuo, 1956).

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