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Microsurgical Techniques for Transplantation of Organs Containing Lymphoid Tissue

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Summary

The technical methods of transplantation of spleen, small bowel and hind extremity in rats used in our laboratory have been presented. Vascularized spleen orthotopic and heterotopic grafts and small bowel transplants are used for studies on spontaneous migration of isotope labelled cells from and through these organs, hind extremity transplants serve as a source of live bone marrow tissue.

The development of techniques of vascularized grafts in small animals of organs containing lymphoid tissue as spleen, bowel, lymph nodes and extremities, has opened the possibilities to study the kinetics of spontaneous release and migration of lymphoid cells from these organs to the lymphoid and non-lymphoid tissues, to the site of allograft or foci of inflammation. It also enabled the investigations on the in vivo response of transplanted lymphoid tissue in the host-versus-graft and graft-versus-host reactions. Lymphoid cells can be labelled in the lymphatic tissues with isotopic and other types of markers and transferred with the transplanted whole organ to the syngeneic recipients. Then, the spontaneous release of these cells and their anatomical location in various tissues may be followed. This gives an insight in the process of dissemination of immune information throughout the body by the cells.

Spleen transplantation in the rat

The technique of spleen transplantation in the rat has been primarily worked out by *Lee* (5) and slightly modified in our laboratory.

Procurement of the donor

Under ether anesthesia, the abdomen is shaved cleansed with alcohol and entered through a midline incision. The celiac artery and its tributaries: the hepatic, left gastric and splenic arteries are identified. The two first are doubly ligated and divided. Then, the pancreatic tissue is dissected from the splenic pedicle and all vessels running to the stomach ligated and divided. The splenic vein is traced down to the portal vein. The superior mesenteric vein is tied and divided distal to the confluence of the splenic vein, and the portal vein cleaned. After the aorta had been dissected and tied distal to the celiac axis, a clamp is placed on the aorta about 1 cm above the celiac artery. An injection needle is inserted into the aortic segment with celiac artery and 5 ml of cold heparin saline (20 u/ml) slowly injected to wash out blood from the spleen. An aortic-celiac-splenic segment is obtained by dividing the aorta below the ligature and the clamp (Fig. 1). In case orthotopic spleen transplantation is planned, a rather long segment of aorta should be taken, since the distance between the arterial and venous anastomoses will be rather long. A segment of the wall of the portal vein with its splenic branch is excised (Fig. 1). The spleen is placed in 4 °C saline solution. Depending on the purpose of the study, its weight is estimated, if it contains labelled cells the radioactivity is measured, or the spleen is perfused with isotope labelled compounds to mark the cellular subpopulations.

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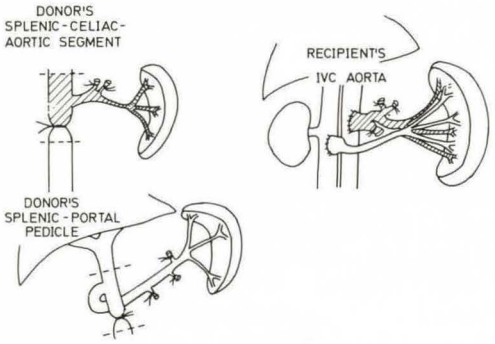

Fig. 1 Schematic drawing of the technique of heterotopic spleen transplantation in the rat (according to *Lee* et al. (5)

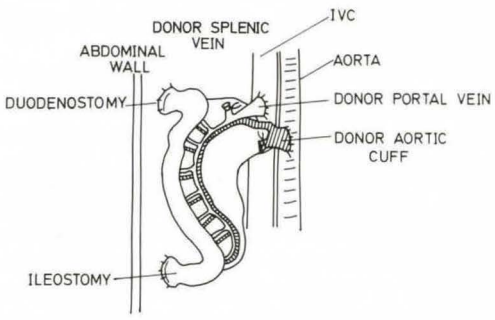

Fig. 2 The diagram of the technique of heterotopic transplantation of small bowel in rat (according to *Monchik* et al. (6)

Procurement of the recipient

Under ether anesthesia the abdomen is opened and the aorta and inferior vena cava dissected free from the adjoining tissues. If a heterotopic spleen transplant is planned both vessels are clamped longitudinally with rubber-covered vascular clamp and a 2 mm opening made in the anterior walls. In case of an orthotopic grafting only the aorta is clamped, but the portal vein dissected.

Transplantation of the spleen

The aortic celiac-splenic segment is anastomosed end-to-side to the recipient's aorta with No. 8-0 dermalon* and the portal-splenic segment with No. 8-0 or 7-0 dermalon end-to-side to the IVC. The hilum of the spleen is fixed with a suture to the neighbouring tissues to prevent rotation of the splenic pedicle. For an orthotopic transplantation, the portal-splenic segment is anastomosed end-to-side to the dissected portion of the portal vein of the recipient.

Functional evaluation of the transplanted spleen

Blood flow in the grafted synergetic spleen, 24 hr after transplantation, is usually slightly higher than in the controls or in spleens left *in situ*. In our studies it was 0.49 ± 0.22, 0.22 ± 0.08 and 0.24 ± 0.01 percent of the cardiac output, respectively. Phagocytic function of the syngeneic spleen transplant, measured with 99 m Tc-colloid injected intravenously, remains within normal limits or may be slightly increased (1).

Measuring of the spontaneous release from the transplanted spleen of different populations of cells migrating physiologically through or originating in that organ is also valuable for functional evaluation of the spleen graft. We employ for that purpose our own method (8) of transplanting syngeneic spleens containing cells labelled with isotopic markers. Briefly, the population of the thoracic duct lymphocytes or peripheral blood mononuclear cells is labelled *in vitro* with ^{51}Cr and injected i.v. into a syngeneic recipient. About 12 to 20 percent of the labelled cells accumulate within 24 hr in the spleen. That spleen is grafted to another syngeneic recipient. Labelled cells contained in the spleen leave that organ and their location in other tissues can be measured quantitatively in the course of time. About 67 % of radioactivity of the thoracic duct lymphocytes is retained in the transplanted spleen after 24 hr, but 12.4 ± 3.3 goes to other tissues. The lymphoid tissues receive 6.9 ± 1.53 % or radioactivity, the non-lymphoid tissues 5.52 ± 1.4 %. When the release of blood mononuclear cells from the transplanted spleen is studied, 6.37 ± 1.46 of radioactivity is found in lymphoid and 14.15 ± 2.3 in non-lymphoid tissues. Non-functioning spleen transplants loose within

*Daves & Geck, American Cyanamid Co.

24 hr more than 50% of radioactivity and only traces can be recovered in the lymphoid tissues.

Small bowel transplantation in the rat

The technique of heterotopic small bowel transplantation has been worked out by *Monchik* and *Russell* (6) and of orthotopic transplants by *Kort* et al. (4). The transplanted bowel may serve as model for studies on migration of lymphocytes from lamina propria and *Payer's* patches to other tissues and to the lumen of the gut. Above 6–10 percent of i.v. injected isotope labelled lymphocytes home within 24 hr in the bowel, among them a large number of lymphoblasts. Also kinetics of the traffic of lymphocytes from the lymphoid tissues to the bowel can be studied in rats receiving bowel transplants at various times after i.v. administration of labelled cells.

Heterotopic small bowel transplantation. Under ether anesthesia, the abdomen is opened in the midline, the bowel retracted to the left and the retroperitoneal space entered. The aorta at the origin of the superior mesenteric artery is dissected. The tributaries of the portal vein from the mesenteric vein up to the liver hilum, including the gastric and splenic vein are ligated. The mesenteric vein is separated from the pancreas. The distal duodenum and terminal ileum are divided and the lumen of the bowel irrigated with 50–100 ml of 0.5% neomycin sulphate solution. The aorta is divided above the level of origin of the superior mesenteric artery, the portal vein divided at the liver hilum. The mesenteric lymph node can be removed, if necessary, after dividing the mesenteric peritoneum, without ligating small blood vessels. The bowel is perfused through the artery with 25 ml of 4 °C heparin saline (20 u/ml), using a plastic cannula. In the recipient the abdominal aorta and IVC are separated from the adjoining tissues. The vascular anastomosis is performed as described for spleen transplantation (Fig. 2). The ends of the transplanted bowel are exteriorized and sutured to the abdominal wall as duodenostomy and ileostomy.

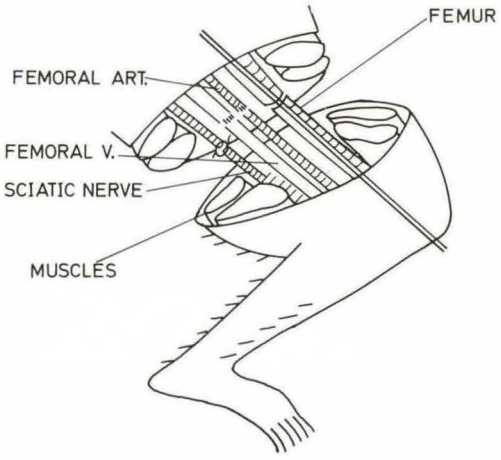

Fig. 3 The diagram of the technique of hind-limb transplantation in the rat

Orthotopic small bowel transplantation

After opening of the abdomen of the recipient the bowel is retracted to the left and the aorta below renal vessels freed. An anastomosis between donor's aortic-mesenteric segment and recipient's aorta is carried out. The portal vein of the donor is anastomosed end-to-side with the portal vein of the recipient. During that procedure the superior mesenteric artery of the host is clamped to avoid intestinal congestion.

Both ends of the transplanted bowel are anastomosed with the appropriate ends of recipient's duodenum and ileum. Then the host's small bowel is removed.

Transplantation of limbs in the rat

The technique of limb replantation and transplantation has been worked out by *Buncke* et al. (2, 3) and modified by *Shapiro* (7). Transplanted syngeneic or allogeneic limb in the rat may serve the studies on spontaneous migration of bone marrow cells to other tissues, immune reactions in the transplanted bone marrow and changes produced by the transplanted bone marrow tissue in the recipient.

Procurement of the donor limb

Under ether anesthesia, a circumferential mid-thigh skin incision is made. The proximal skin flap is raised and the epigastric vessels ligated. Using sharp scalpel, the muscle groups are divided. Hemostasis is maintained. The sciatic nerve is identified, labeled with a suture, and divided. An injection of 1 % xylocain is given beneath the femoral sheath, and all branches of the femoral artery and vein doubly ligated and divided. The femur is cut with a jeweler's hacksaw, so as not to crush the bone. The artery and vein are ligated close to the inguinal ligament and cut sharply distally. A cannula is introduced to the artery and approximately 50 ml of 4 °C heparin saline (20 u/ml) injected slowly. Then the limb is placed in a cold saline solution.

Procurement of the recipient

All the initial steps of the procedure are the same as in the donor. However, when dissecting the femoral vessels microvessel clamps are applied to the artery, proximal to the origin of the epigastric and to the vein proximal to the profunda, leaving enough room for anastomosis. The stumps of vessels should remain rather long.

Transplantation of the limb

Bone fixation is achieved first with an intramedullary rod made from a 22-gauge needle (Fig. 3). The femoral vein and then the artery are anastomosed with a 10-0 dermalon suture under operating microscope. The sciatic nerve can be repaired if necessary with epineural No. 10-0 dermalon suture. The muscles are approximated with No. 4-0 sutures. If patency of the anastomosed vessels can be demonstrated after 10–15 min, the wound is closed. A cylindrical metal splint can be applied to the skin around the limb, or an adjustable yoke around the rat's neck, to prevent from carnivorous autoamputation of the anesthetic limb.

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