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ORTHOTOPIC LIVER TRANSPLANTATION IN THE MOUSE

Heart and kidney transplantation in the mouse has been well established (1, 2), but orthotopic liver transplantation has not been reported. Although the mouse is one-tenth the size of the rat, there are many advantages to using the mouse for immunologic research. The mouse genome has been more thoroughly characterized than the rat or any other species of mammal. The mouse H-2 system also bears a striking resemblance to the human HLA system. In addition, there are numerous genetically defined inbred strains and wealth of monoclonal antibodies that are commercially available for mouse investigations.

A key development in rat orthotopic liver transplantation (3) was the introduction by Zimmerman et al. (4) and Kamada and Calne (5), of the cuff technique instead of suture for some of the venous vascular anastomoses. This method shortened the clamping time of the portal vein and increased survival. We have applied this principle to mouse orthotopic liver transplantation. In our pilot studies, more than 40 syngeneic mouse liver replacements were performed before 6 long-term survivors were obtained. The experience reported here is with the next 48 attempts, in which the surgical success rate was 83%.

Male inbred syngeneic mice 10-12 weeks old (25-32 g), from Jackson Laboratory, Bar Harbor, ME, were used as size-matched donors and recipients under methoxyflurane (2,2-dichloro-1,1-difluoroethyl methyl ether) anesthesia. The strains used were B6AF₁ (27 pairs), C57BL/6 (11 pairs), and BALB/c (10 pairs). Clean but not sterile operative technique was used, and all procedures were performed under the operating microscope with 4-6.4× magnification.

Donor operation. After shaving and disinfecting the abdomen with Betadine or alcohol, a complete midline incision was made. Four small retractors and a retracting clamp on the xiphoid process provided exposure. The bile duct was transected. The

pyloric, splenic, right adrenal, and right renal veins, as well as the hepatic artery were ligated. Heparin (100 units) diluted to 0.3 ml with lactated Ringer's solution was slowly injected into the penile vein. After cross-clamping the thoracic aorta and dividing the suprahepatic vena cava close to the diaphragm, the liver was perfused with 1 ml cold lactated Ringer's solution, given through the abdominal aorta. Since the hepatic artery was already ligated, the liver perfusion was from the splanchnic venous return. The liver and surrounding tissues were kept cool with frequent rinses of cold lactated Ringer's solution. The infrahepatic vena cava was divided at the level of the left renal vein, leaving a sufficient length for cuff preparation. The freed liver was then placed into a container of cold lactated Ringer's solution for further preparation. The cystic duct was ligated and the gallbladder removed. The infrahepatic vena cava and the portal vein were cleaned for insertion through Teflon tubes (outer diameter 1.7 mm for vena cava, 1.2 mm for portal vein), using a cuff length of 1.5 mm and an equal vein length, which was folded back and tied (Fig. 1). These steps were facilitated by a stabilizing clamp.

Recipient operation. Through a midline incision, the bile duct, hepatic artery, and right adenal vein were ligated and divided. The liver ligaments were incised and the suprahepatic vena cava was encircled with a ligature, which was used to pull the liver and diaphragm down for application of a Santinsky clamp on the suprahepatic vena cava. When the liver was removed, the infrahepatic vena cava, portal vein, and suprahepatic vena cava were cross-clamped, in that order. Maximum length was retained with the recipient to facilitate the venous reconstructions. The suprahepatic vena cava anastomosis was constructed first with a continuous 10-0 nylon suture (Dermalon, Davis & Geck Inc.). A one-suture anastomosis technique was used to

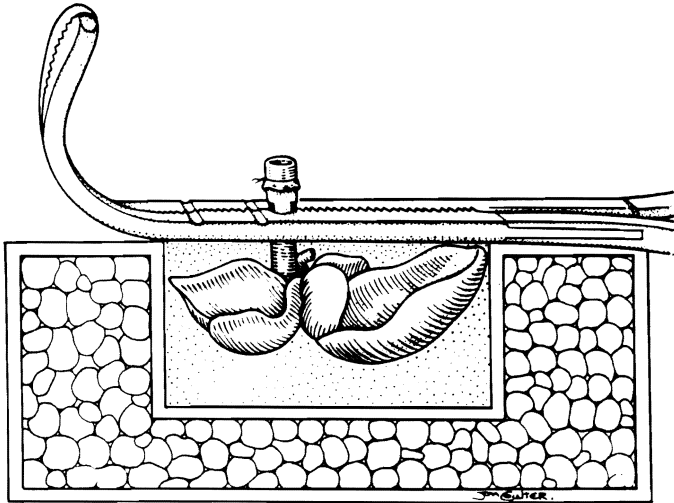


FIGURE 1. Method of venous cuff preparation used for the portal vein and infrahepatic vena cava. Note that the liver is submerged in cold solution during these maneuvers.

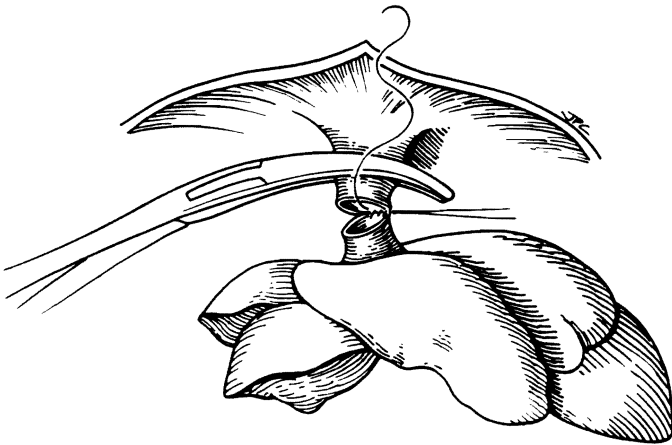


FIGURE 2. Continuous suture anastomosis of the suprahepatic vena cava, using a one-suture technique for manipulation and exposure of the posterior vessel walls.

aid in manipulating and visualizing the posterior anastomotic wall (Fig. 2). Next, the graft portal vein cuff was inserted into a venotomy in the anterior wall of the recipient portal vein which was kept on tension with a clamp and inflated with injected saline. A silk tie was placed to secure the vein over the cuff and the liver was revascularized by removing the suprahepatic and portal venous clamps. Portal venous occlusion was always for less than 20 min. The infrahepatic vena cava cuff was inserted in a similar way, taking care to avoid excessive retrograde bleeding in the process. No attempt at rearterialization was made. Biliary continuity was restored by tying the ducts over a 4-mm polyethylene tube stent (OD of 0.61 mm, Becton Dickinson; Parsippany, NJ). Blood lost during operation was replaced with lactated Ringer's solution. The abdomen was closed in two layers. Single intramuscular injections of 5 mg cefamandol nafate and 60,000 units Bicillin were given and the mice were kept under a warming lamp until they became active. Food and water were allowed ad libitum.

Eight mice were bled from the retroorbital sinus weekly for the first month and monthly thereafter postoperatively for determination of serum glutamic oxaloacetic transaminase, al-

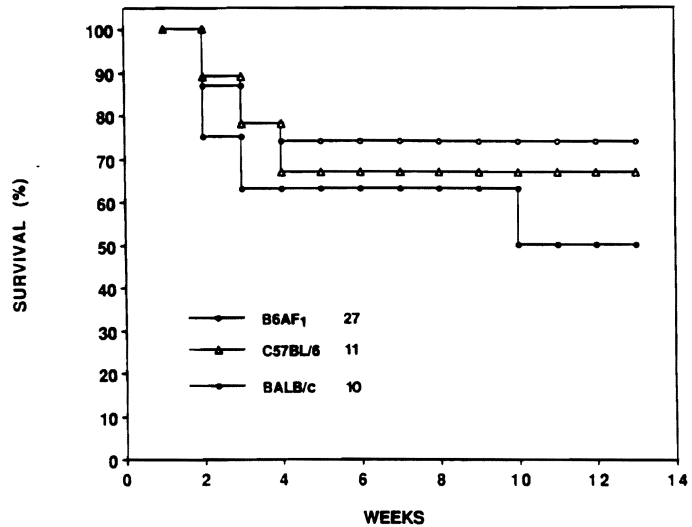


FIGURE 3. Life survival (actual) after syngeneic liver replacement in three strains of mice.

kaline phosphatase, bilirubin, total protein, and albumin. Animals that died from complications of the operation were autopsied, and histopathologic analyses were obtained when the cause of death was not readily apparent. In long-term survivors, tissues for histopathology were obtained by reoperation and wedge biopsy. Tissues were fixed in neutral buffered formalin, embedded in paraffin, sectioned at 4 μ and stained with hematoxylin and eosin.

Of the 48 recipients, 40 (83.3%) lived for more than one week, and 27 (67%) lived more than 100 days. The longest survivors have been followed for more than 12 months. The survival curves were similar in the 3 strains (Fig. 3).

The deaths in the first postoperative week were from technical causes. The causes of death of the 13 recipients that died between 8 and 100 days were bile duct complications (4 examples), peritonitis (3), pneumonia (3), phlebotomy for blood sample collection (2), and unknown (1). The delayed deaths were concentrated in the second and third weeks (Fig. 3), and after this only a single mouse was lost (from a phlebotomy).

Because 2 mice died from the phlebotomies, one early and one late, only 6 provided enough serial samples to be included. The SGOT levels were elevated after transplantation, as were the serum bilirubins, but these returned to or toward normal except in an animal that died of bile duct complications. In contrast to the other biochemical tests, the alkaline phosphatases tended to become worse with time and were above the upper limits of normal in all animals tested for the full 100 days. Total protein and albumin levels were normal except in the animals that died of bile duct complications.

Histopathologic studies showed that mice that died within 3 weeks had areas of scattered coagulation necrosis, most prominently in the hepatic hilum. Abscesses of variable sizes were in or around the infarcted areas. Mononuclear infiltrates were not seen in the viable liver but the liver sinusoids frequently contained neutrophils. The extrahepatic organs were unremarkable.

Two mice had open liver biopsy at 49 and 56 days, and a third was killed at 133 days. Grossly, the livers appeared normal. Histopathologically, the livers had no major changes (Fig. 4), although there were subtle, usually focal, abnormali-

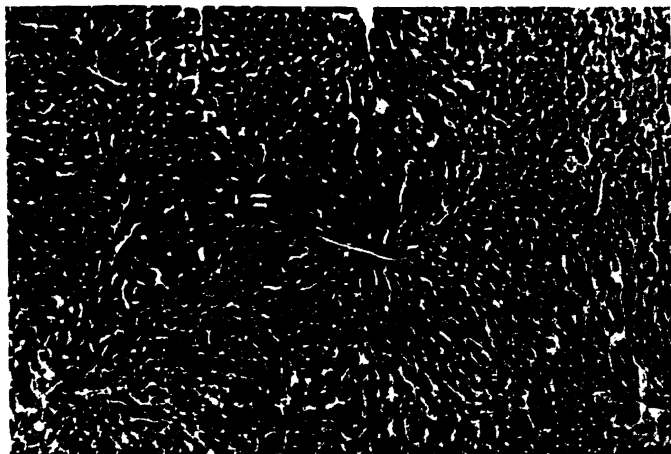


FIGURE 4. Syngeneic liver graft of mouse killed 133 days postoperatively (H&E; original magnification: $\times 40$).

ties. Many of the triads had mild fibrosis and/or mild ductular proliferation with a light lymphoplasmocytic inflammatory infiltrate. The duct epithelium frequently showed apoptosis and vacuolization. Hepatic arteries were difficult or impossible to identify in most of the triads. There was no evidence of cholestasis.

To our knowledge, this is the first description of orthotopic liver transplantation in the mouse. Although the surgical principles and techniques of OLT in the mouse are similar to those in the rat, the procedure is more difficult because of the smaller size. The most troublesome step was the suprahepatic vena cava anastomosis. This was facilitated by using one-suture anastomosis technique for the purpose of manipulation and ensurance of lumen patency. The anastomosis, which required 12–16 min, was performed with continuous 10–0 Dermalon because of the softness and flexibility of this suture. In our developmental series, and in a few of the animals in the definitive series, an anhepatic period greater than 20 min was incompatible with consistent success. This may reflect an intolerance of the mouse to hepatic ischemia, to venous congestion of the splanchnic and systemic systems, or to both. In the rat, an anhepatic period less than 26 min can be routinely tolerated (5).

The lethal surgical complications were from defective revascularization of the portal vein. As with the rat (4, 5), failure to provide an arterial blood supply did not jeopardize long survival. However, degenerative changes in the biliary epithelium and other subtle histopathologic abnormalities in the portal triads may have been the consequence of this omission. It is noteworthy that the canalicular enzyme, alkaline phosphatase, was the one biochemical index that did not become normal during the 100-day period of observation.

Notwithstanding this potential imperfection, the mouse model should be useful in the future study of liver transplantation. Since numerous genetically defined inbred strains—including congenic strains of mice—are available, immunologic studies should be possible with greater precision than has been

feasible until now. The resulting new information obtained may help to explain why the liver is privileged relative to other organs and why it may have a protective effect on other concomitantly transplanted organs (5). The availability of well-defined monoclonal antibody reagents will allow tracer studies and identification of cells involved in allorecognition and rejection or the tolerance induction which is incongruously easy after liver transplantation (6). The prospect of evaluating monoclonal antibodies for therapeutic purposes is equally inviting. Finally, several unique liver disease models, such as mouse viral hepatitis, that are analogs of human disorders, can be studied in greater detail (7, 8).

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