the presence of antiallotypic antibodies in the patient's serum can be obtained by testing the serum against a panel of lymphocytes. The use of a panel of lymphocytes frozen and stored in Terasaki tissue typing trays was found to expedite such screening (*8*). It is being suggested that quick screening should be carried out for all prospective recipients to estimate the probability of a positive crossmatch against a random donor. By using lymphocytes frozen in Terasaki trays, a quick screening could be carried out for all patients within a matter of about 5 hr. A patient reacting with 50% of the panel members has roughly a 50% chance of having a positive crossmatch with a random donor. It may, at times, not be possible to wait for the crossmatch results before transplantation; under such circumstances, knowing the probability of a positive crossmatch against a random donor may facilitate the decision for undertaking or not undertaking a transplant. We are in agreement with the recommendation of Weil et al. (*5*) that cardiac transplantation should not be done in the presence of a positive, warm T cell crossmatch unless the patient is not likely to survive long enough to wait for another organ.

In summary, we have described a patient who had cytotoxic antibodies to 50% of the members of a random panel and had a positive, warm T cell crossmatch with the donor lymphocytes. The cardiac transplant suffered transient vasculitis, but did not undergo hyperacute rejection. Evidence for humoral rejection, i.e., positive direct immunofluorescence and positive crossmatches, waned despite initial positivity. Humoral and cellular rejection were present simultaneously in the initial period, but were discordant after the initial 2 weeks. It is suggested that quick screening for lymphocytotoxic antibodies be carried out using lymphocytes frozen in Terasaki typing trays to determine the likelihood of a positive crossmatch. Whenever possible, transplantation in the face of a positive crossmatch should be avoided.

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VALUE OF A PHYSIOLOGICAL LIVER TRANSPLANT MODEL IN RATS
INDUCTION OF SPECIFIC GRAFT TOLERANCE IN A FULLY ALLOGENEIC STRAIN COMBINATION[1]

With the one known exception of spontaneous graft tolerance, DA → PVG (*1, 2*), fully allogeneic rat liver transplantation (RLT) still shows very poor results, whereas in more than one semiallogeneic combination specific transplantation tolerance has been achieved (*3, 4*). RLT, whether performed in an auxiliary heterotopic model (*4*) or in variously modified orthotopic models (*1, 5–7*), has, so far, not yet been immunologically analyzed after reanastomosis of the hepatic artery. Major complications after RLT, such as biliary peritonitis and bile duct necrosis, may be attributable to the lack of arterial blood supply.

Improving the modified orthotopic technique described by Lee et al. (*8*), by reanastomosing the hepatic artery, syngeneic LEW and fully allogeneic BN livers were grafted into LEW recipients. In comparison of the results of transplantations performed with reanastomosis of the hepatic artery (REART) to those performed under nonrearterialized (Non-REART) conditions, we will show: (1) dramatic increase of isograft survival using the optimized technique, (2) improvement of graft morphology, (3) elimination of unspecific cell-mediated in vitro reactivity, and (4) specific transplantation tolerance in the fully allogeneic BN → LEW combination.

By using the inbred rat strains LEW ($RT1^l$) and BN ($RT1^n$), the following four combinations were studied: LEW → LEW Non-REART, $n = 22$, REART, $n = 17$ and BN → LEW Non-REART, $n = 8$, REART, $n = 12$. RLT (Non-REART) was performed with the modified orthotopic Lee technique (*8*) using a polyethylene cuff for bile duct anastomosis. In REART rats in addition to suprahepatic inferior vena cava anastomosis, portal vein anastomosis and anastomosis of the infrahepatic inferior vena cava, the aorta of the donor, bearing the celiac axis and the hepatic artery, was anastomosed in an end to side fashion to the recipient's infrarenal aorta. Mean operation time for Non-REART rats was 45 min, for REART rats 70 min. Survival rates were evaluated and morphological studies of the liver (open liver biopsies were taken at day 10 and then in 4-week intervals) were performed in every surviving animal. Cell-

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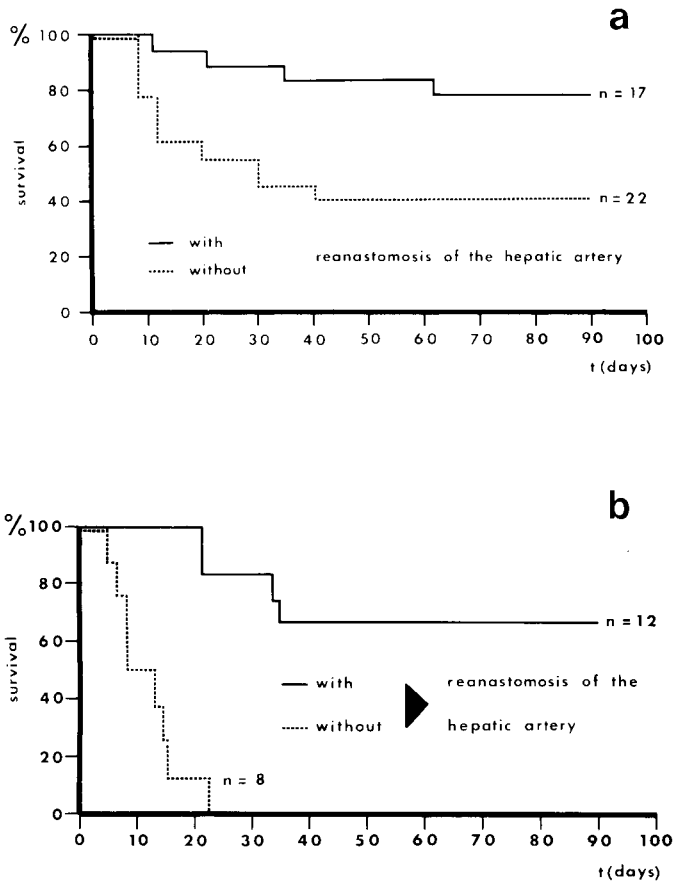

FIGURE 1. Survival of liver graft recipients with or without reanastomosis of the hepatic artery. a: syngeneic group LEW → LEW; b: fully allogeneic group BN → LEW.

mediated cytotoxicity (CMC) using LEW fibroblasts as targets was tested with the MCA (*9*), comparing reactivity in the blood, spleen, peripheral, and mesenteric lymph nodes and Peyer patches. Tolerance was tested by grafting donor (BN) and third-party (F344) strain skin onto long-term survivors.

The following results were obtained.

Survival rates. In the syngeneic LEW → LEW combination, survival of liver graft recipients greatly improved from 45% in the Non-REART group to 80% in the REART group for the 90th day (Fig. 1a). Four LEW rats bearing REART isografts are still alive and well at 240, 256, 280, and 360 days postoperatively. In the fully allogeneic BN → LEW combination, survival of REART recipients was 70% beyond day 90, while every one of the Non-REART rats died by day 22 postoperatively (Fig. 1b), showing severe signs of graft rejection. Bile duct necrosis and biliary peritonitis, which were very common in the Non-REART syngeneic as well as in the nonsurviving Non-REART allogeneic graft recipients, and which often proved to be fatal complications, completely disappeared in the REART individuals of both experimental groups.

Graft morphology. Fibrosis of the liver and massive periportal infiltrations seen in Non-REART syngeneic recipients disappeared completely in REART rats (Fig. 2). Even in long-term survivors the syngeneic transplant could not be distinguished from a normal liver. In contrast to strong periportal and intrahepatic mononuclear cell infiltrations in the Non-REART BN → LEW group, such infiltrations were restricted to the periportal field in the REART group and decreased constantly after day 14 postoperatively.

Cell-mediated reactivity. Unexpectedly, the Non-REART syngeneic LEW rats showed strong unspecific cell-mediated in vitro reactivity in blood, spleen, peripheral, and mesenteric lymph nodes and Peyer patches, when tested at day 25 postoperatively against syngeneic LEW fibroblasts. When the REART syngeneic LEW recipients were tested in the same way, it was seen, that CMC was reduced to zero in all lymphatic compartments. Thus, the basis for in vitro determination of specific alloimmune reactivity appears to be set and experiments analyzing CMC in REART LEW recipients with BN allografts are in progress.

Transplantation tolerance. Four long-term survivors in the REART BN → LEW group accepted donor-specific (BN) skin grafts (transplanted on day 50 postoperatively) unlimitedly, while rejecting third-party skin (F344) in the normal way (mean = 11.8 ± 0.4 days). Thus, reanastomosis of the hepatic artery allows for specific transplantation tolerance in a fully allogeneic strain combination, which so far has been known to be tolerant only in the related semiallogeneic system (*4*).

Reanastomosis of the hepatic artery in orthotopic RLT, on the one side, demanding the most intensive training and skill of the microsurgeon, or improvements in cuff techniques for venous anatomoses as described by Limmer et al. (*6*) and Miyata et al. (*7*) on the other side, are two different approaches aiming at the same target: prevention of more or less fatal nonimmunological processes after orthotopic RLT in isograft recipients in order to be able to study immunological and morphological parameters successfully in allograft recipients.

The results obtained in LEW rats with REART isografts are striking: The increase of survival is about 100% compared with the reports of Limmer et al. (*6*) for two syngeneic groups, WAG and PVG. Biliary peritonitis and necrosis, which, according to Kamada and Calne (*5*) are far too often causes of graft failure, are completely eliminated. The histological finding that the REART isograft does not differ from the normal liver is an indication of an intact organ structure, which is necessary for an unimpaired function. The elimination of unspecific CMC allows an unbiased analysis of the specific status of liver allograft recipients.

It may be pointed out that specific transplantation tolerance could be achieved in the fully allogeneic BN → LEW combination only on the basis of the microsurgical improvements reflected by the morphological and functional data presented above.

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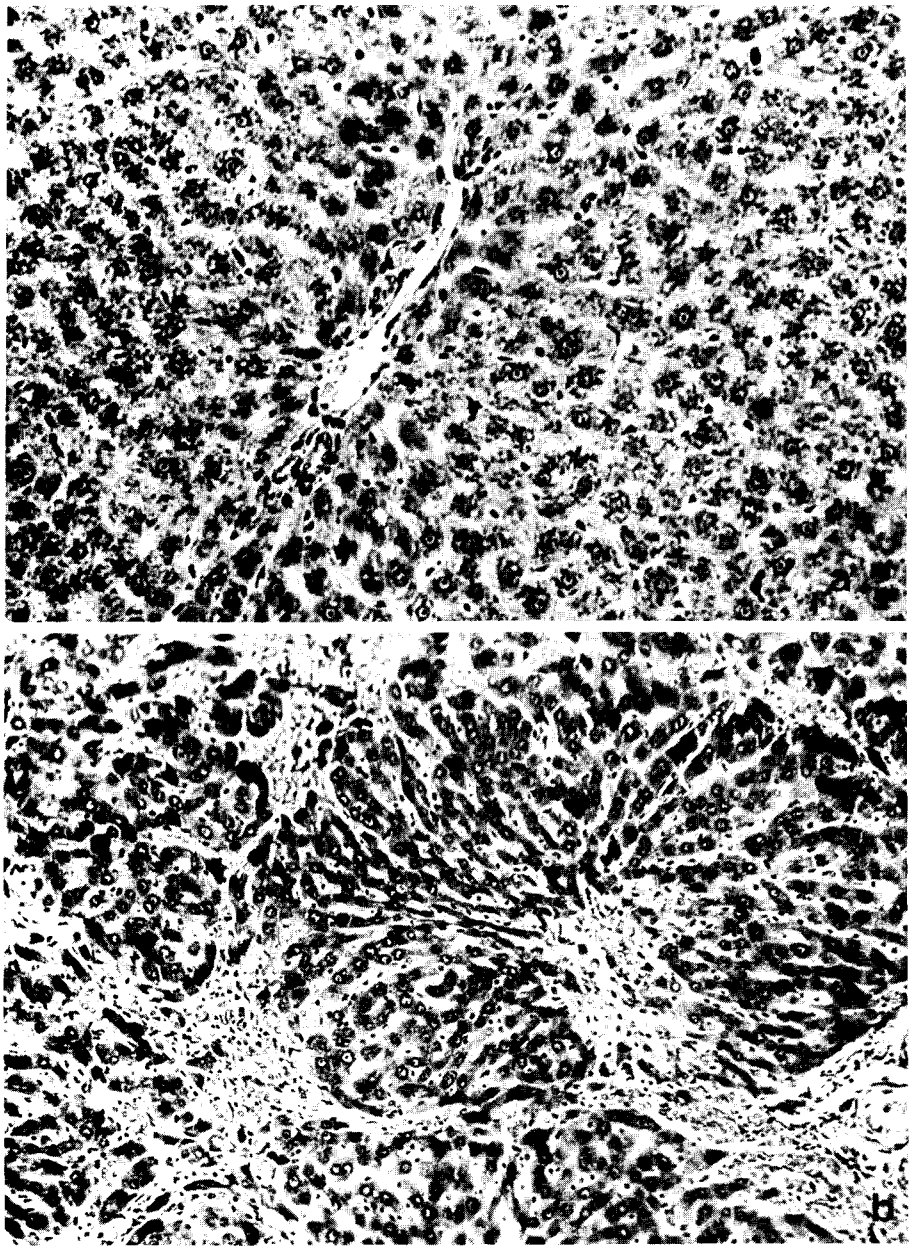

FIGURE 2. Morphology of syngeneic liver grafts with (a) or without (b) reanastomosis of the hepatic artery. a: complete disappearance of fibrosis and intrahepatic mononuclear cell infiltrations; b: liver fibrosis and massive intrahepatic mononuclear cell infiltrations.

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