SUCCESSFUL HAMSTER-TO-RAT LIVER XENOTRANSPLANTATION UNDER FK506 IMMUNOSUPPRESSION INDUCES UNRESPONSIVENESS TO HAMSTER HEART AND SKIN[1]

In addition to its well-known resistance to antibody (*1*) and cell-mediated (*2*) rejection, the liver allograft can confer these advantages to other tissues and organs transplanted concomitantly or subsequently from the donor or donor strain (*3, 4*). We report here an example of this same hepatic tolerogenicity after hamster livers were transplanted to rats under FK506, ameliorating thereafter the otherwise intractable rejection of hamster heart and skin xenografts.

Before performing these "shielding" experiments, we first confirmed and extended our earlier observations (*5, 6*) of the liver xenograft's privileged state relative to other organs. Full-thickness hamster skin grafts were debrided of fatty tissue, sutured onto the chest of Lewis (LEW, $RT1^l$) rat recipients, inspected daily after the 6th day, and scored as rejected on the first day of total epithelial necrosis. Hamster heart grafts were placed in the abdominal cavity by anastomosing the graft aorta and pulmonary artery end-to-side to the recipient's abdominal aorta and inferior vena cava, respectively (*6*); rejection was defined by cessation of the heartbeat on abdominal palpation. Liver grafts replaced the recipient's own organ and were revascularized with portal venous blood only (*6*); graft rejection was defined as the time of the animal's death.

In these preliminary experiments, hamster hearts in untreated rat recipients were destroyed by xenospecific antibodies in 3±0.0 (SD) days, whereas livers survived this initial insult and were rejected by combined humoral and cellular rejection at 7.0±0.5 days, one day later than full-thickness grafts of skin (6.0±0.7) (Fig. 1A). When the rats were treated daily with the T cell–directed immunosuppressant FK506, heart xenograft survival was not prolonged by FK506 and the effect on skin grafts was minimal. In contrast, liver xenograft survival time was increased 10-fold, with 30% of the liver recipients living >100 days (Fig. 1B).

As reported elsewhere (*6*), microvascular platelet/fibrin thrombi, hemorrhage, and necrosis caused by antibody rejection in the heart and liver xenografts were associated with vascular binding of immunoglobulins (IgM > IgG) that contemporaneously rose dramatically in serial plasma samples. In the untreated liver recipients, splenomegaly was invariable by the time of death at 6–7 days. However, under FK506, splenomegaly was not prominent and heterophile antibody titers that rose initially as in untreated animals declined to baseline levels after reaching a peak on the 5th or 6th day. In selected liver xenograft recipients under FK506, sequential biopsies during the first 30 days showed self-resolving humoral, then humoral-cellular, and finally predominantly cellular rejection.

The first invading immunocytes in treated or untreated recipients were predominantly $OX8^+/OX19^+$ (cytotoxic T), and NKR-$P1^+$ (natural killer) cells. In contrast to the typical localization of mononuclear infiltrates to the portal triads of allografts, these cells were distributed throughout the hepatic sinusoids (*6*). The cells disappeared in the surviving xenografts under FK506, and in later samples it was shown with immunophenotypic detection techniques that chronically surviving grafts always had extensive replacement of donor Kupffer and dendritic cells by those of the recipient (*7*). The cell repopulation and graft chimera formation were similar to that which occurs in accepted liver allografts (*8, 9*). The other histopathology of long-surviving xenografts ranged from normal to various stages of rejection. The most common cause of late graft failure was intra- or extrahepatic biliary obstruction.

The surviving liver recipients from the foregoing preliminary experiments were used for shielding experiments. LEW rats bearing hamster livers for 40–50 days under daily FK506 had their immunosuppression stopped for 2 weeks on the day of skin or cardiac transplantation from third-party (outbred) hamsters or from C3H mice. These animals (Table 1, group 3) freely accepted skin and cardiac grafts from third-party hamsters. At the same time, they retained the same ability to reject C3H mouse skin and heart xenografts as that possessed by control rats that had had drug pretreatment only (Table 1, group 2). These LEW ($RT1^l$) recipients also rejected skin allografts from ACI ($RT1^a$) donors in 11–13 days (n=5). To rule out the possibility that the results were due in part to residual immunosuppression from the prior chronic FK506 therapy, control LEW rats without liver transplantation were pretreated for 30 days with 1 mg/kg/day FK506 before test heart or skin xenotransplantation, after which no treatment was given. When transplanted alone, survival of the hamster skin was prolonged an average of 3.0 days by the 30-day pretreatment ($P<0.01$) but survival of the hamster heart xen-

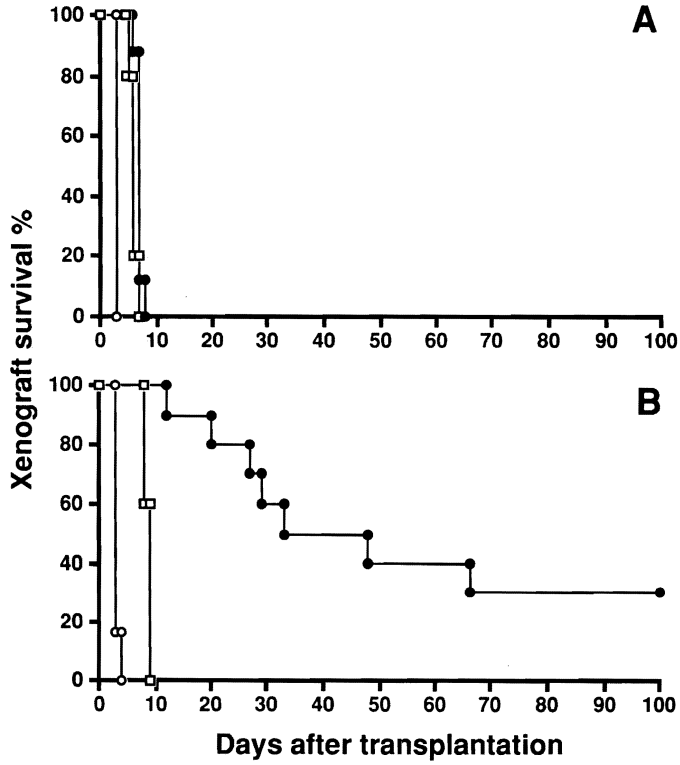

FIGURE 1. Hamster-to-rat xenotransplantation (A) Graft survival in untreated controls; skin grafts (open square [n=5]), heart grafts (open circles [n=6]); and liver grafts (closed circles [n=8]). (B) An intramuscular injection of 1 mg/kg/day FK506 was given daily for the first 30 posttransplant days and half this daily dose thereafter until day 100. Symbols as in (A): skin grafts (n=5), heart grafts (n=6), and liver grafts (n=10).

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TABLE 1. Results of hamster and mouse skin or heart xenotransplantation to LEW rats 40–50 days after successful xenografting of hamster liver (OLT)

Recipient treatment	Survival days			
	Skin graft		Heart graft	
	Hamster	C3H mice	Hamster	C3H mice
None	5,6,6,6,7	6,6,7,7,8	3,3,3,3,3,3	2,2,3,3
FK506 (1 mg/kg/day ×30)	8,9,9,9,10	9,10,10,10,10	3,3,3,4	2,2,2,3
Prior OLT and daily FK506[a]	22[b],33,62[b],69 73,78,78,89	9,10,11,12	54,60[b],73,120	2,3,3,3

[a] The protocol of FK506 treatment is described in the legend to Figure 1. When skin or heart grafts were grafted between 40 and 50 days after liver transplantation, immunosuppression was stopped for 2 weeks and then restarted at 0.5 mg/kg every other day up to day 100.

[b] Recipients died of biliary obstruction with grafts intact.

TABLE 2. Evidence that LEW rats accept equally liver donor and 3RD-party hamster skin engrafted on the day of liver xenotransplantation

OLT	FK506[a]	Skin donor	Skin graft survival
No	No	Hamster	5,6,6,6,7
No	Yes	Hamster	8,8,9,9,9,
Yes[b]	Yes	Hamster liver donor	15[c],40[c],54[c],60[c],>130
Yes[b]	Yes	Third party hamster	15[c],40[c],54[c],60[c],>130

[a] Immunosuppression was started on the day of transplantation, as described in the legend to Figure 1.

[b] Skin and liver graft survival were synonymous.

[c] Recipients died of biliary obstruction with grafts intact.

ografts was the same as in the untreated controls. Mouse skin ($P<0.01$), but not mouse hearts, also had slight prolongation of survival after recipient pretreatment (Table 1, group 2).

The lack of strict donor specificity of the liver-induced tolerogenicity to the hamster organs was studied further in 5 LEW rats who were given 2 full-thickness skin grafts on the day of liver xenotransplantation under FK506—one from the liver donor, and the other from a third-party hamster. All 10 skin grafts were accepted for as long as the liver recipients survived, in contrast to invariable skin graft rejection in non-liver recipients given the same immunosuppression (Table 2). In hamster-to-hamster allograft controls, skin grafts in non-immunosuppressed animals were rejected in 13.1±4.2 (SD) days (n=6, range 11–16 days). Although the relatively long skin survival suggested that there was a good average histocompatibility within the Syrian hamsters, the experiments eliminated the possibility that the hamster colony was inbred to a syngeneic state.

These experiments have shown that a multifaceted immunologic privilege enjoyed by the liver in the moderately difficult hamster-to-rat xenograft model is qualitatively similar to that in hepatic allotransplantation models. The hamster-to-rat species combination can be categorized as "semidiscordant" because of the xenospecific hyperacute (antibody-mediated) rejection of the heart, but not the liver, that occurs after 3 days. Numerous explanations have been advanced to explain the liver's relative ability to withstand an antibody insult (*10*) including the protection to its microvasculature provided by a double blood supply (not relevant in our experiments in which the livers were not arterialized); its large microvascular surface available for antibody absorption; its secretion of new donor-specific soluble class I antigens (*11*) or IgG types (*8*) that theoretically could neutralize preformed antigraft antibodies; and the special qualities of its sinusoidal bed. This last possibility has had a special appeal because of the discovery by Porter in 1969 that the Kupffer cells and macrophages lining the sinusoids are quickly replaced in all successful hepatic grafts with recipient cells although the hepatocytes permanently retain their donor specificity (*8*).

It is now suspected that this cell repopulation, which also includes lymphoid and dendritic cells, is a generic phenomenon critical to the "acceptance" of any organ allograft and responsible as well for hepatic tolerogenicity (*9*). The extent to which the seeding of donor cells and consequent systemic microchimerism occur depends on the immunologic substrate (especially the amount of bone marrow–derived dendritic cells) available in the donor organ for cell peripheralization. The liver is the most richly endowed of the solid organs with these cells. Achievement of the cell repopulation requires potent immunosuppression with agents like FK506 during the cell transition. In xenotransplant models, it also depends on the avoidance of antibody rejection, which is more easily accomplished with the liver than other organs. The reason why the hepatic tolerogenicity that ensues is species- but not individual-specific may be the genetic similarity of hamsters even when they are outbred. This demonstration of hepatic tolerogenicity in a xenograft model could have clinical implications.

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