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

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, RT1l) 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 invariably 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 extrapleural 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 (RT1l) recipients also rejected skin allografts from ACI (RT1l) 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 xenografts always had extensive replacement of donor Kupffer and dendritic cells by those of the recipient.

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).

1 This work was supported by Project Grant DK 29961 from the National Institutes of Health, Bethesda, MD.

Reprinted from transplantation
Volume 55, Number 3, March 1993
Copyright © 1993 by Williams & Wilkins
FK506

660

liver transplantation, immunosuppression was stopped for 2 weeks and then restarted at

Prior

None
described in the legend to Figure 1.

ografts was the same as in the untreated controls. Mouse skin
tolerogenicity to the hamster organs was studied further in 5

sinusoids are quickly replaced in all successful hepatic grafts
with recipient cells although the hepatocytes permanently
retain their donor specificity (8).

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 histocompati-
bility within the Syrian hamsters, the experiments eliminated
the possibility that the hamster colony was inbred to a
specific soluble class I antigens (11) or IgG types (8) that
available for antibody absorption; its secretion of new

FK506 (1 mg/kg/day ×30)

Prior OLT and daily FK506*

8,9,9,9,10

22?,33,62,69

73,78,78,89

*(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.

Recipient treatment

Survival days

<table>
<thead>
<tr>
<th>Recipient treatment</th>
<th>Skin graft</th>
<th>Heart graft</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin graft</td>
<td>Heart graft</td>
<td></td>
</tr>
<tr>
<td>Hamster</td>
<td>C3H mice</td>
<td>Hamster</td>
</tr>
<tr>
<td>None</td>
<td>5,6,6,6,7</td>
<td>6,6,7,7,8</td>
</tr>
<tr>
<td>FK506 (1 mg/kg/day ×30)</td>
<td>8,9,9,9,10</td>
<td>9,10,10,10,10</td>
</tr>
<tr>
<td>Prior OLT and daily FK506*</td>
<td>22?,33,62,69</td>
<td>9,10,11,12</td>
</tr>
<tr>
<td>Survival days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*(Recipients died of biliary obstruction with grafts intact.

**Skin and liver graft survival were synonymous.

These experiments have shown that a multifaceted immu
nologic 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 spe
cies combination can be categorized as "semidiscordant" be
cause of the xenospecific hyperacute (antibody-mediated) re
jection 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 antigen antibodies;
and the special qualities of its sinusoidal bed. This last possi
bility 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 re
tain 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 respon
sible as well for hepatic tolerogenicity (9). The extent to which
the seeding of donor cells and consequent systemic microchi
merism 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 immuno
suppression 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 tolerogenic
ity 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.

Luis A. Valdivia*
Anthony J. Demetris*
John J. Fung*
Susanna Celli*
Noriako Murase*
Thomas E. Starzl*

1 The Pittsburgh Transplant Institute and the Department of Surgery,
2 The Pittsburgh Transplant Institute and the Department of Surgery,
3 The Department of Pathology.
4 Address reprint requests to Thomas E. Starzl, M.D., Ph.D., De
partment of Surgery, 3601 Fifth Ave., 5C Falk Clinic, University of
Pittsburgh, Pittsburgh, Pennsylvania, 15213.

REFERENCES

rents to orthotopic liver transplantation, with special reference to
survival, resistance to hyperacute rejection, and biliary duct

Received 14 May 1992.
Accepted 16 June 1992.