Clinical xenotransplantation


Abstract: Two baboon liver xenografts transplanted to patients with B virus hepatitis supported life for 70 and 26 days but did not function optimally despite minimum or no histopathologic findings of overt humoral or cellular rejection in serial biopsies. However, there was evidence of complement activation in both cases, which in retrospect was thought to explain the unsatisfactory outcome. Strategies to deal with this problem are discussed.

Introduction

In June 1992 and January 1993, we made attempts at baboon-to-human liver xenotransplantation, both of which failed after 70 and 26 days. The patients whose original disease was B hepatitis had no evidence of viral reinfection during their posttransplant survival, and there was little histopathologic evidence of conventional humoral or cellular rejection of these livers. The clinical courses have been published in detail elsewhere [1,2], including the infectious complications that were the immediate causes of death: ruptured mycotic intracerebral aneurysm caused by aspergillus in Case 1, and peritonitis, secondary to an anastomotic leak at the jejunojejunostomy of the Roux-y biliary reconstruction, in Case 2. However, the underlying reason for failure in both cases was suboptimal xenograft function, which has not been satisfactorily explained.

Surgical procedures

The techniques were adapted from hepatic allograft transplantation and seemed satisfactory. The body weights of the baboon donors were only 40% of those of the recipients, necessitating the so-called piggyback operation that leaves the recipient vena cava intact (Fig. 1). The livers regenerated up to optimal volume for recipient size in both cases. Biopsies showed multitudes of proliferating hepatocytes and duct cells. There were almost no infiltrating immunocytes. The interesting possibility has been raised recently by the studies of Shiraishi et al. [3] that the use of small-to-large liver allografts (and by inference xenografts) unwittingly introduces an augmented antigenicity factor. In such circumstances, the increased MHC Class II expression that is independently associated with liver regeneration [4] appeared to be responsible for much more severe cellular and humoral rejections than seen with size-matched liver transplantation in the same rat strains. Murase had independently made the same observations in our laboratory (Table 1).

Preoperative status

The conventional lymphocytotoxic crossmatch of the recipient sera with their donor lymphocytes was positive initially but negative after dithiothreitol (DTT) treatment, meaning that the antibodies were largely IgM. Postoperatively, even the conventional crossmatches became negative. Both patients had...
Fig. 1. Piggyback operation used for both baboon-to-human xenotransplantations.

donors of their own blood type, A to A in Case 1, and B to B in Case 2. They were immune competent with in vitro testing, although Patient 1 had asymptomatic HIV infection, and had undergone posttraumatic splenectomy 3 years previously. This patient was half the age (35 years) of Patient 2 and far less frail. Patient 2 had developed deep hepatic coma and was on a ventilator.

Postoperative courses

The first patient awoke promptly from anesthesia, resumed diet and ambulation, and became jaundice-free for most of the 70 days of survival. However, his alkaline phosphatase was elevated from the second week onward, suggesting partial biliary obstruction. At autopsy after 70 days, the entire biliary tree was filled with inspissated bile, and most of the biliary ducts, which by this time had become bile lakes, were denuded of epithelium.

The second patient never woke from his pre-existing coma, and in contrast to the first one failed to become anicteric. Again, the allograft at autopsy was filled with inspissated bile. Having the same cholestatic problem twice without clear evidence of mechanical obstruction raised the possibility that the baboon liver produced a lithogenic bile in the human environment. Although this kind of metabolic incompatibility has not been absolutely ruled out, it does not seem likely in view of other physiologic evidence of hepatic dysfunction. Neither patient was able to maintain a postoperative serum albumin above 2 gm%. Both patients developed renal failure.

Pathologic observations

The disparity between the paucity of the histopathologic abnormalities, and the discouraging functional deficiencies of these transplants suggested incomplete control of xenograft rejection. Nearly 30 years ago, six baboon kidney xenografts were transplanted to patients treated with azathioprine-prednisone immunosuppression. The organs functioned for 6 to 60 days [5]. At the end, the baboon kidney xenografts had fierce cellular rejection. However, the key finding was a presumably antibody-mediated (humoral) occlusive endothelitis of the graft vessels that choked off much of the arterial supply. The consequent distal ischemia appeared to be responsible for patchy gangrene of the xenografts, interspersed between islands of still-functional parenchyma. Similar gross and histopathologic findings were reported more than 20 years later by Bailey after cardiac xenotransplantation under a cyclosporine-based immunosuppressive regimen (the Baby Fae case) [6].

In contrast, the two liver xenografts treated with a four-drug immunosuppressive cocktail (FK 506, prednisone, prostaglandin E₁, and cyclophosphamide) had little or no evidence of cellular rejection in biopsies obtained over the 70 and 26 days of sur-

Table 1. The effect of 70% donor hepatectomy 1 day before liver transplantation

<table>
<thead>
<tr>
<th>Donor → Recipient</th>
<th>Donor hepatectomy (−24 hours)</th>
<th>n</th>
<th>Survival (days)</th>
<th>Median (days)</th>
</tr>
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<tr>
<td>LEW → BN</td>
<td>No</td>
<td>10</td>
<td>23, 23, 23, 25, 28</td>
<td>28.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>29, 29, 30, 32, 37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>5</td>
<td>7, 8, 9, 11, 12</td>
<td>9.0</td>
</tr>
</tbody>
</table>

*The recipient animals received no immunosuppression. The role of rejection was established by histopathologic studies, and by control experiments showing that the hepatectomy effect could be abolished by a single dose of FK 506 (1 mg/kg IM) on the day of transplantation.*
The question of complement activation injury

In both patients, total complement was depleted for most of the critical first 2 weeks while complement components C3, C4, and C5 became undetectable [1]. During this time, circulating immune complexes appeared. This complement evolution was similar to that reported previously in recipients of allografts transplanted across a positive lymphocytotoxic crossmatch [8]. After 10 days, the complement system settled down but irreversible damage may have occurred. Although the xenografts had little evidence of cellular or vascular rejection, they exhibi­ted a very fine microsteatosis on their first biopsies that became obvious within a few days, particularly in Case 2 [1,2]. This finding has been reported in cases of allotransplantation with inexplicable primary hepatic nonfunction. Although these findings receded, the microsteatosis is suspected to represent a sublethal injury that precluded long term success in either case. There was prompt binding of IgM and IgG in the grafts. The IgM but not the IgG largely disappeared from the graft tissues in later biopsies.

The relationship of these findings to those of hyperacute allograft rejection has not been proved, but we believe that these livers were acutely damaged by an incomplete version of the kind of rejection first described in ABO incompatible kidney recipients [9] and subsequently in patients with a positive lymphocytotoxic crossmatch [10,11]. Although these syndromes usually are associated with anti­body antibodies, this is not an absolute requirement [12,13]. The antibody versus nonantibody distinction in cases of hyperacute rejection described in the first instance the classical pathway of complement activation in which the first steps are antibody­dependent, and in the second, the alternative pathway, which does not require an antibody trigger or the participation of complement components C1, C2, and C4. The consequent hyperacute allograft rejection syndromes with or without preformed antigraft antibodies are not thought by us to be fundamentally different from those seen after xenotransplantation of organs between genetically diverse spe­cies [1,2]. We postulate that our baboon xenografts suffered damage by similar but less obvious mecha­nistic events.

Control of complement activation: Back to the laboratory

Anticomplement strategies

The complement pathogenicity that derives from the cleavage products of C5 and C6 has been effectively mitigated (but not eliminated with cobra venom (which depletes C1 and C2), and soluble recombi­neric nonparenchymal cells (NPCs). In addition to the liver, Patient 2 was given a large dose of unpurged bone marrow cells (3 x 105/kg body weight) peroperatively. He also had mixed xenogeneic chimerism at all times until death.
activation [16]. K76 has been used with another
drug called FUT (a synthetic inhibitor of serine pro-
teases) that had a small therapeutic effect by itself.
K76 and FUT together allowed guinea pig heart
graft survival in rats for 100 min [16].

We obtained a small supply of K76, which, when
tested by Dr. Noriko Murase, gave superior results
to those reported by Miyagawa et al. [16]; Miya-
gawa et al. had administered the drug intraperito-
neally rather than intravenously. Median survival of
guinea pig hearts in rats was increased by a single
dose of 200 mg/kg from 8 min to more than 8 hr,
and in one experiment to more than a day [2]. The
surprising potency of intravenous K76 by itself or
in combination with the other drug FUT has also
been demonstrated in the very difficult pig-to-dog
kidney xenograft model. Survival out to 7 hr was
obtained, during which 200 mg/hr of urine was pro-
duced. However, in both the rodent and large an-
imal xenograft models, the effect was limited to a
few hours.

Brief control of the complement pathogenicity
with any of the drugs or agents now available is not
significant clinically. However, these drugs could be
an adjunct to other strategies. One ancillary strat-
egy could be the production of transgenic animals
whose organs contain transfected human genes that
regulate complement activation. This was a subject
of intense interest at the recent international xe-
notransplant meeting in Cambridge.

Complement change after experimental liver transplantation

The need to prevent complement activation may be
short-term, particularly if the transplantation is of
the liver, which is the principal source of the body’s
complement and the sole source of most comple-
ment components such as C3. A paper by Valdivia at
the Cambridge meeting has potential importance
because it has demonstrated the species restriction
of complement [17].

In Valdivia’s investigation, the combination of
hamster liver and its complement plus the rat recipi-
ent equaled a rat transferred to a hamster comple-
ment environment (Fig. 2). Valdivia showed how,
in the new complement environment, hyperacute
rejection of stable liver and heart xenografts could
be precipitated with a small dose intravenous anti-
hamster rat serum (Fig. 3). This was demonstrated
to be a specific effect of the rat complement rather
than the rat anti-hamster antibody because the hy-
peracute rejection could be completely avoided by
simply heating the injected serum at 56°C for 30
min (Fig. 3). This allowed retention of the rat anti-
hamster antibodies but removed the rat complement.
This species restriction of complement is a discov-
ery that may be exploitable to facilitate xenograft
acceptance.

Fig. 2. Demonstration by Valdivia et al. [17] that the comple-
ment environment of a xenograft recipient becomes that of the
donor species. In these experiments, the dominant complement
of rat recipients of hamster livers become hamster-specific
within a few days, a change that was permanent. See text for
further explanation.

Conclusions

The xenograft barrier may be more vulnerable
than most people realize at present. If comple-
ment activation proves to be the explanation,
breaking this barrier whether it involves the clas-
smal or alternative pathway may not be unrealis-
tic. Once this is achieved, it seems clear that
conventional cellular and vascular rejection can
be controlled with modern drug combinations.
The complement reactions are the same as those
that can abruptly destroy allografts. At present,
the most intriguing question is whether the pro-
duction of transgenic animals whose organs have
complement regulatory proteins (such as DAF and
CD59) will be helpful.

Acknowledgments

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Fig. 3. Second stage experiments of Valdivia et al. [17] following the engraftment shown in Figure 2, showing species restriction of complement activation. Rats bearing long surviv-
ing hamster livers hyperacutely rejected these xenografts when injected with rat anti-hamster serum, but not if the serum was first decomplemented by heating.

References