Although two men recently given baboon liver xenografts subsequently died, the cases provided valuable information on the effects of xenotransplantation in humans. Here, Tom Starzl and colleagues discuss these cases and the prospects for future attempts at baboon-to-human xenotransplantation.

Human liver xenotransplantation

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On 28 June 1992 and on 10 January 1993, two men aged 35 and 62 years, respectively, with end-stage chronic active hepatitis caused by hepatitis B virus (HBV) had their diseased livers replaced with baboon hepatic xenografts. As well as the worldwide shortage of donor organs, these efforts were prompted by evidence that the baboon liver would be resistant to the HBV that reinfects most allografts under comparable circumstance[1].

Comparison of cases

Extensive infectious surveillance was performed. Both donors were healthy and were antibody-positive for foamy virus, Epstein–Barr virus (EBV), cytomegalovirus (CMV), and simian agent 8; in addition, donor 1 had antibodies against varicella zoster virus. The donor/recipient weights were 25.8/70 kg in case 1 and 35.8/80.4 kg in case 2. The orthotopic transplantations were with the piggy-back technique. Postoperatively, there was histopathologic evidence of rapid regeneration[1] and a

tripling or quadrupling of graft volume within 3–4 weeks. The conventional lymphocytotoxic crossmatch of both recipient sera to their donor lymphocytes was positive in both cases but negative after dithiothreitol treatment.

Both patients had ABO-compatible donors: A to A in case 1 and B to B in case 2. However, there were differences that might have had an impact on the efficacy of perioperative immune modulation. Patient 2 was nearly twice as old and far more frail. He did not have splenectomy until the fourth postoperative day whereas patient 1 had had a splenectomy 3 years previously. Both patients were immunocompetent, although the first also had an human immunodeficiency virus (HIV) infection.

Patient 1 awoke promptly from anesthesia, resumed diet and ambulation, and was jaundice-free for most of the 70 days of survival. Tests of liver injury had only minor abnormalities. Prothrombin time and other clotting factors promptly normalized except for thrombocytopenia. Hypoalbuminemia (<2 gm%) prompted human albumin infusions throughout[1].

In contrast, patient 2 remained icteric (Fig. 1) and comatose after the operation. Patient 1 developed renal failure after 21 days, and patient 2 became anuric immediately after the transplantation.

The cause of death in both cases was multifactorial, the proximate premortem events being a ruptured mycotic aneurysm (*Aspergillus*) of a cerebral artery in patient 1 and peritonitis in patient 2 secondary to an anastomotic leak at the jejuno–jejunostomy of the Roux-y biliary reconstruction.

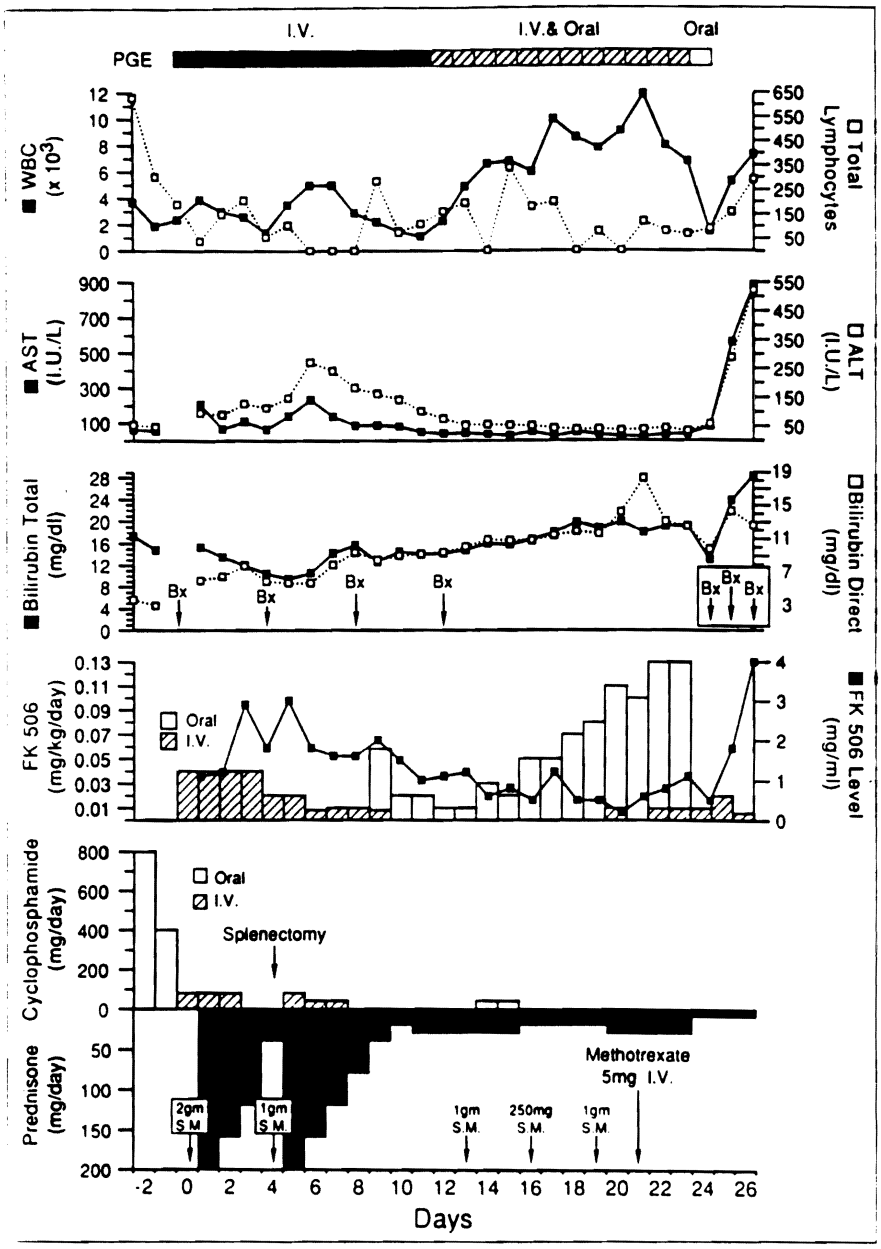

FIGURE 1

Clinical course of second baboon liver recipient. SM, solumedrol® (methlyprednisolone); PGE, prostaglandin E; BX, biopsy.

Questions about rejection

The two recent cases were not in *terra incognita* except for the fact that the liver was the xenograft. Nearly 30 years ago, it was shown that baboon kidney xenograft rejection in humans under azathioprine-prednisone immunosuppression[2] resembled the renal allograft rejection that occurs in recipients who have preformed anti-donor antibodies[3]. The six transplanted baboon kidneys in the earlier experience survived 6 to 60 days. In all six there was fierce cellular rejection plus a presumably antibody-mediated (humoral) occlusive endotheliolitis of the graft microvasculature[2]. In turn the distal ischemia caused by the vascular injury appeared to be responsible for patchy gangrene of the xenografts, interspersed between islands of still functional parenchyma. Similar histopathologic findings were reported more than 20 years later after cardiac xenotransplantation under a cyclosporine-based immunosuppressive regimen[4].

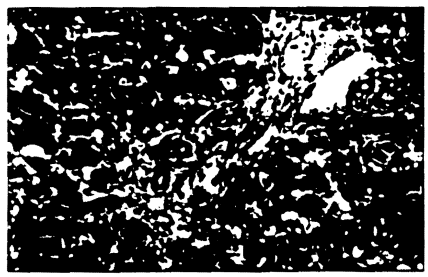

FIGURE 2

Liver xenograft biopsy from patient 2, obtained on day 23 (the same day as the repair of the intestinal anastomotic leak), showed no evidence of rejection. Note intact bile ducts and cholestasis (315 × original magnification, H&E).

Effectiveness of the FK506-based drug cocktail

The cellular as well as humoral (vascular) rejection that destroyed all of the earlier baboon kidney and heart grafts was prevented in the two recent liver xenografts with a four-drug cocktail of FK506, prednisone, perioperative prostaglandin E_1, and a short course of cyclophosphamide[1]. The value of cyclophosphamide and other anti-metabolites in the interdiction of the xenospecific vascular injury had been seen in hamster-to-rat heart and liver transplants[5,6]. After breaking through the antibody barrier in the animal model, chronic treatment with FK506 or cyclosporine alone allowed the rat recipients of both organs to survive indefinitely.

Because it was suspected that the first recipient had been over-immunosuppressed, mainly as the result of a 40-day course of cyclophosphamide[1], the latter drug was given in lower doses for only 10 days in patient 2 (Fig. 1). When the jaundice remained at an unsatisfactorily high level, a dose of 5 mg intravenous methotrexate was given on postoperative day 21, with the assumption that an immunologic process was responsible for the cholestasis.

Vascular rejection

In the first of the two liver xenograft recipients, six samples of the graft between the time of transplantation and autopsy on the 70th postoperative day were entirely free of arteritis associated with vascular rejection[1]. Although there was a positive conventional crossmatch preoperatively, and binding of IgM and IgG absorption and platelet sludging in the sinusoids by the xenograft immediately after reperfusion (as shown by direct immunofluorescence), the immunoglobulins were largely absorbed from the graft tissues in the following days and weeks, except for IgG, which was positive throughout. The second patient had eight xenograft biopsies from 0 to 26 days, including the autopsy. The findings from these specimens were similar to those in case 1, with no evidence of rejection-related arteritis.

Cellular rejection and hepatitis

One of the biopsies obtained from patient 1 on day 12 had a mild focal cellular rejection by the conventional criteria used for hepatic allografts, while no definite evidence of cellular rejection was seen in any of the biopsy samples from patient 2 (Fig. 2). The biopsy obtained on day 64 from patient 1 showed a mild but diffuse increase in T ($CD3^+$) and natural killer (NK; Leu-7^+) cells in the sinusoids and septal bile ducts, but the findings were insufficient for an unequivocal diagnosis of rejection. Immunoperoxidase staining revealed no evidence of reinfection of the hepatic allograft with HBV in either case.

Unexplained cholestasis and microsteatosis

A few T cells (CD4 < CD8) and even fewer NK cells in small bile ductules were given special attention because of the unexplained finding of intrahepatic cholestasis in the livers, which otherwise were normal (except for diffuse fine fatty infiltration). Although patient 1 was not jaundiced for nearly 2 months after the transplant, the canalicular enzymes (serum alkaline phosphatase and gamma-glutamyl transpeptidase) were elevated astronomically (maximum 10000 international units for the alkaline phosphatase) from the second week onward. When icterus finally developed 55 days after transplantation, it was ascribed to partial obstruction of the reconstructed bile duct, even though the biliary-enteric anastomosis appeared satisfactory by cholangiography[1]. At autopsy, 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. This can be the end result with unrelieved obstruction, but the alternative explanation is that the epithelial damage was the primary rather than a derivative event.

A mechanical explanation for the cholestasis has been undermined by experience with the second recipient, whose xenograft showed the same microsteatosis and cholestasis on biopsy after 4 days despite an unquestionably adequate biliary anastomosis. This patient's pre-existing icterus was not relieved, the lowest bilirubin level being 8 mg/dl on the fourth postoperative day. Bilirubin levels, which had fallen from 17.3 mg/dl, increased thereafter to the terminal concentration of 28.3 mg% (Fig. 1). In this patient, as in the first one, the jaundice was incompletely responsive to boluses and increased maintenance doses of prednisone. However, bile collected from an indwelling catheter abruptly improved in color and consistency after some of the steroid bolus treatments.

The liver as an optimal xenograft

Immunologic advantages

In the search for drugs that would interdict the xenograft reaction after hamster-to-rat transplantation, two organs were used routinely for screening. One was the heart, which is considered immunologically difficult because it undergoes antibody rejection in unmodified rat recipients within 3 days[5,6]. The other organ was the immunologically privileged liver[5], which is easier to protect from rejection than the heart, shields concomitantly transplanted organs from rejection, including xenografts, and resists the attack of preformed xenospecific antigraft antibodies[7]. Because the liver presumably would not contribute to its own rejection, one factor in the antibody resistance could be its synthesis of most of the complement components[1].

In addition, most of the immunologic advantages of the liver have been linked to the high concentration in the liver of potentially migratory dendritic cells and other sessile tissue leukocytes that leave the transplanted hepatic graft and produce systemic chimerism in the course of allograft acceptance and tolerance induction[8]. In the hamster-to-rat experiments[9] and in patient 1 (Ref. 1), chimerism was proved to be the same as seen in allografts. Consequently, an effort was made in patient 2 to augment the natural tolerogenicity of the liver with an infusion of baboon bone marrow cells shortly after revascularization of the liver. PCR (polymerase chain reaction) analysis revealed chimerism in all subsequent blood samples from patient 2.

Metabolic questions

In spite of its immunologic advantages, it remains to be determined if the baboon liver is an appropriate organ for human xenotransplantation trials. The possibility has not been disproved that a baboon liver could impose on a human

recipient a lethal interspecies metabolic difference. So far, there has been no direct evidence of this in connection with hepatic-based clotting factors and metabolites such as albumin, several globulins, uric acid and cholesterol. Nevertheless, the baboon liver was incapable of supporting more than 2 gm% serum concentration in the human environment. In addition, the cholestatic findings in the two patients could in some way be the product of a metabolic incompatibility rather than having an immunologic etiology.

The hypoalbuminemia in both of the recipients was not considered surprising at the time because both of the baboon donors had serum albumin concentrations in the 2–3 gm% range. These animals were accepted primarily because of their large size, even though they were both more than 15 years old. This may have been a mistake. There is no evidence for a genetically imposed limit on albumin production by the baboon liver, and it is not clear why the albumin of the donors was so low since the other synthetic hepatic functions, as well as the histopathologic features of the livers, were normal. Nevertheless, it is clear that hypoalbuminemia is not a 'normal' finding in this colony of domestically bred and reared animals. In a recent (unpublished) study of 25 ostensibly healthy baboons housed at the Southwestern Foundation for Biomedical Research, San Antonio, the mean serum albumin was 3.5 ± 0.7 SD gm/dl (range 1.8–5.1).

Table 1. Levels of total serum complement (CH100), complement components (U/ml) and immune complexes

Days postop	CH100 (>60)	C3 (83–177)	C4 (15–45)	C5 (6–20)	IC
1	<21	ND	ND	ND	+
2	<21	ND	ND	ND	+
4	<21	ND	ND	ND	+
6	<21	ND	ND	ND	
8	<21	ND	ND	ND	-
9	<21	ND	ND	ND	-
11	21	ND	ND	ND	-
14	43	ND	ND	ND	-
17	55	64	17	20	-
23	66	59	15	17	-
26	61	51	15	11	+ (low)
28	44	40	13	14	-
33	55	58	14	15	+ (low)
64	55	ND	ND	ND	-

From Ref. 1. ND, not detectable.

The baboon liver xenograft and beyond

Additional liver transplant cases are not planned until the issue of metabolic compatibility is resolved. If metabolic incompatibility is the problem, the liver xenotransplantation experience will have provided a signal for a trial with an organ with simpler functions, such as the kidney or heart.

However, we do not recommend further human trials with any organ at present. We need to investigate the possibility that these two livers were damaged by a mechanism similar to the Shwartzman or local Arthus reaction – independent of classical rejection pathways. Nearly 25 years ago, working with Frank Dixon, we drew attention to the similarity of hyperacute renal rejection in patients with or without antibodies to the Shwartzman and Arthus reactions, which depend upon complement activation with neutrophil participation[10,11]. Since then, it has been shown that the complement pathogenicity is derived from the stimulatory action of anaphylactotoxins (formed by the cleavage of C3 and C5) on mast cells and leukocytes – giving rise to a variety of proinflammatory responses. Efforts to inhibit individual inflammatory mediators, such as platelet-activating factor, to prevent hyperacute xenograft rejection have been only marginally successful[12].

The harmful consequences of cleavage of C3 and C5 to these anaphylactotoxins is mitigated by complement receptor 1 (CR1), which binds to the complement fragments. This knowledge of the complement cascade prompted the T-Cell Sciences Corporation, a biotech company in Cambridge, Massachusetts, to develop a recombinant soluble human complement receptor (type 1) that prevents both the classical and alternative complement pathways by binding to the anaphylactotoxins and promoting their degradation to inactive forms. By shutting off the inflammatory mediators, the recombinant complement receptor can interdict the Arthus reaction, a simulated Shartzman reaction, and neutrophil-mediated tissue injury[13–15].

In the next critical step, this recombinant molecule was shown, at relatively non-toxic doses, to delay the hyperacute rejection of heart allografts in highly sensitized recipients – prolonging survival from 3 to 32 hours[16]. The same drug in high doses prolonged xenograft survival in an extremely difficult guinea-pig-to-rat model from 17 minutes to more than 12 hours[17].

Control of the complement pathogenicity could be the missing piece in the treatment regimen used for our clinical xenotransplantations. In our first patient, Manez and Kelly found depleted total complement for the critical first 2 weeks while complement components C3, 4 and 5 were undetectable (Table 1). During this time, circulating immune complexes appeared. After this, the complement system settled down. This is the target of the complement receptor, which is envisaged as an additional constituent of the drug cocktail used in our two clinical cases. Experiments to test this hypothesis are underway in experimental models.

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