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Baboon-to-human liver transplantation

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Our ability to control both the cellular and humoral components of xenograft rejection in laboratory experiments, together with an organ shortage that has placed limits on clinical transplantation services, prompted us to undertake a liver transplantation from a baboon to a 35-year-old man with B virus-associated chronic active hepatitis and human immunodeficiency virus infection.

Liver replacement was performed according to conventional surgical techniques. Immunosuppression was with the FK 506-prednisone-prostaglandin regimen used routinely for hepatic allotransplantation, to which a daily non-myelotoxic dose of cyclophosphamide was added. During 70 days of survival, there was little evidence of hepatic rejection by biochemical monitoring or histopathological examination. Products of hepatic synthesis, including clotting factors, became those of the baboon liver with no obvious adverse effects. Death followed a cerebral and subarachnoid haemorrhage that was caused by an angioinvasive aspergillus infection. However, the underlying cause of death was widespread biliary sludge that formed in the biliary tree despite a seemingly satisfactory choledochojejunostomy. During life and in necropsy samples, there was evidence of the chimerism that we believe is integral to the acceptance of both xenografts and allografts.

Our experience has shown the feasibility of controlling the rejection of the baboon liver xenograft in a human recipient. The biliary stasis that was the beginning of lethal infectious complications may be correctable by modifications of surgical technique. In further trials, the error of over-immunosuppression should be avoidable.

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Introduction

Previous attempts to transplant seven baboon kidneys[1,2] and two hearts[3,4] resulted in graft loss or patient death between 0 and 60 days after transplantation. A common difficulty was uncontrolled cellular rejection, together with antibody-mediated occlusive endotheliolitis of graft microvasculature and parenchymal necrosis.[4,5] Recent laboratory investigations have shown that the presumably humoral component of xenograft rejection could be diminished by a short course of antimetabolite therapy, such as cyclophosphamide, which targeted the B-cell proliferative response.[6-8] By overcoming this antibody barrier, the value of maintenance therapy with T-cell-directed immunosuppressants was unmasked.[6-8]

We now describe a baboon-to-human liver xenotransplantation in which FK 506 and cyclophosphamide were given as immunosuppressants, together with prednisone and prostaglandin, both of which help to mitigate preformed antigraft antibody syndromes and cellular rejection.[9,10]

Patient and methods

Recipient history

A 35-year-old white male had a history of abnormal liver function tests since 1984 with recurrent bleeding from oesophageal varices and haemorrhoids which began 2 years later. Hepatitis B virus (HBV) and human immunodeficiency virus (HIV) had been diagnosed in 1987. When his spleen was ruptured and removed after a motorcycle accident in 1989, his prothrombin time (PT) was

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TABLE I—DONOR AND RECIPIENT BIOCHEMISTRY

—	Albumin (g/L)	Total protein (g/L)	Cholesterol (mmol/L)	PT (s)	Alkaline phosphatase (IU/L)	Uric acid (μmol/L)
Donor baboon	18	48	1·03	10·9	387	<30
Patient pre-op	20	63	2·33	16·3	158	481·8
Patient 5 days post-op	20	52	1·16	12·5	452	208·2
Patient 45 days post-op	19	40	1·71	11·5	2812	<30

PT, prothrombin time.

15·7 s, aspartate aminotransferase (AST) 105 IU/L, alanine aminotransferase (ALT) 73 IU/L, albumin 2·7 g/L, and total bilirubin 47·9 μmol/L. Macronodular cirrhosis of the liver was noted at the time of splenectomy, and a biopsy specimen confirmed the clinical diagnosis of chronic active hepatitis. After being refused liver transplantation elsewhere, he came to Pittsburgh in January, 1992, with jaundice, spider naevi, ascites, peripheral oedema, episodic encephalopathy, and deteriorating hepatic function. Hepatitis A, C, and delta were negative. Hepatitis B surface antigen (HBsAg) was positive and antibodies to hepatitis B core antigen were also present; e antigen was negative. There was serological evidence of previous infection with Epstein-Barr virus (EBV), cytomegalovirus (CMV), and herpes simplex virus (HSV).

His clinical condition worsened between January and May, 1992, and he eventually required continuous hospital care. Because the baboon liver was thought to be resistant to HBV infection (J. Hoofnagle, National Institutes of Health, personal communication), baboon-to-human liver xenotransplantation for HBV hepatitis was already under discussion by the Institutional Review Board of the University of Pittsburgh and members of US government agencies. Although the HIV carrier state was an undesirable factor, the patient was accepted into the HBV xenotransplantation protocol because of his urgent clinical status. Prophylactic antiviral therapy with ganciclovir was started, but hyperimmune anti-B virus globulin was not given.

Donor surveillance

The 15-year-old male baboon (*Papio cynocephalus*), who had the same A blood group as the recipient, was obtained for organ donation from the Southwest Foundation for Research and Education, San Antonio, Texas. Retrovirus antibody screen revealed the animal to be negative for Simian T-lymphotropic virus, human T-cell leukaemia virus 1 and 2, Simian immunodeficiency virus, Simian retrovirus 1, 2, and 5, and HIV 1 and 2. He was antibody positive for foamy virus, and had evidence of previous infection with EBV, CMV, Simian agent 8, and Varicella-Zoster virus. Antibodies directed against herpes simplex virus and herpes B virus were not present. In addition, there was no evidence of infection with HBV, HAV, HCV, Marburg virus, encephalomyocarditis virus, lymphochoriomeningitis virus, and haemorrhagic fever virus.

Donor biochemical tests that fell outside normal human ranges included alkaline phosphatase 387 IU/L, serum uric acid <0·5 mg/dL, serum cholesterol 1·03 mmol/L and serum albumin 18 g/L (table I). These values are normal for the baboon.

Transplantation and postoperative course

Liver replacement was done on June 28, 1992, by conventional techniques including venovenous bypass.[12] The 600 g liver from the 25·8 kg baboon donor was preserved with University of Wisconsin solution for 137 min of cold ischaemia. It was inserted by the piggy-back technique into the 70 kg recipient whose excised liver weighed 1750 g. Xenograft venous outflow was through the transplant suprahepatic vena cava into a cuff of the left and middle veins of the excised native liver, which drained into the intact retrohepatic inferior vena cava of the host. The xenograft coeliac axis and portal vein were anastomosed to the recipient common hepatic artery and left portal vein, respectively, after oversewing the right portal branch. Biliary reconstruction was with a roux-en-Y choledochojejunostomy. 20 units of blood were given during the 11 h operation. Generalised wound bleeding secondary to pre-existing coagulopathy (PT 16·3 s) diminished substantially after the xenograft was revascularised.

FK 506, prednisone, and prostaglandin are given routinely after liver allotransplantation (fig 1).[13] Except for higher doses of FK 506 given during the first 2 postoperative weeks, the doses of all 3 drugs were within standard therapeutic ranges. In addition, non-myelotoxic doses (25 to 175 mg/day) of cyclophosphamide were begun 2 days preoperatively and continued for 55 days (fig 1). All four drugs were changed from the intravenous to the oral route when diet was resumed. Doses of intravenous prostaglandin were 0·4 to 1·0 μg/kg per hour PGE_1 and for the oral form were 6 μg/kg per day PGE_2 (misoprostil). From days 12 to 21, prednisone was stopped except for a 1 g bolus of methylprednisolone.

Postoperative investigations

Biochemical studies—Standard liver and renal function tests were repeated daily. In addition, the arterial ketone body ratio, which estimates hepatic mitochondrial integrity and liver energy charge,[14] was monitored daily for the first 30 days. Attention was paid especially to tests whose normal ranges were strikingly divergent in the baboon and human: protein electrophoresis, albumin, alkaline phosphatase, uric acid, and cholesterol.

Immunological studies—Complement-dependent cytotoxicity was measured in pretransplant and post-transplant patient serum samples that were tested against lymphocytes from either the donor baboon or a panel of third-party human donors. Serum samples were tested with and without dithiothreitol (DTT) treatment, which inactivates IgM, thus permitting detection of IgG.[15] Donor

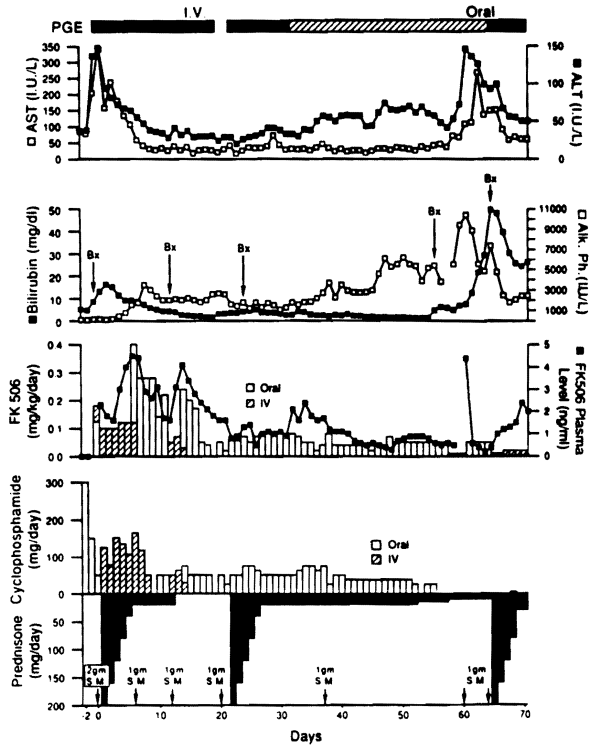

Fig 1—Clinical course after baboon liver transplant.

SM, Solumedrol (methylprednisolone); PGE, prostaglandin E; Bx, biopsy; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Alk Ph, alkaline phosphatase.

TABLE II—POST-TRANSPLANT INFECTIOUS COMPLICATIONS

Postoperative day	Infection	Organism	Treatment
7	Wound infection and bacteraemia	Staphylococcus	Vancomycin and debridement
27	Bacteraemia	Staphylococcus	Vancomycin
29	Oesophagitis	Candida albicans	Amphotericin
30	Viraemia	Cytomegalovirus	Ganciclovir
42	Oesophagitis and viraemia	Cytomegalovirus	Ganciclovir
55	Bacteraemia	Enterococcus faecalis	Vancomycin
56	Bacteraemia	Staphylococcus	Vancomycin
61	Sepsis	Presumed cholangitis after percutaneous transhepatic cholangiogram	Amikacin and imipenem/cilastin
68	Sputum	Aspergillus flavus	Amphotericin
70	Death	Disseminated aspergillus (at necropsy)	

baboon lymphocytes were isolated at the time of transplantation from lymph nodes and spleen, and were cryopreserved in tissue culture media supplemented with 20% pooled baboon sera. Dilutions of patient sera were made to 1 in 512 for pre-transplant samples and 1 in 32 for post-transplant sera. Rabbit complement for crossmatch testing was absorbed with baboon red cells. Pooled human sera and pooled baboon sera were used for positive and negative crossmatch controls, respectively. Daily samples were collected from the patient, batched and tested every three days in the first month post-transplant.

For mitogen response assays, peripheral blood lymphocytes from heparinised blood samples of the xenograft recipient were isolated by a Ficoll-Hypaque gradient and were resuspended at a concentration of 5×10^4 cells per well for 72 h at 37°C with either concanavalin A (ConA) (4 μg/ml) or phytohaemagglutinin (PHA) (10 μg/ml).

Unidirectional mixed lymphocyte reaction (MLR) cultures were set up with 5×10^4 responder (patient) and 5×10^4 irradiated baboon lymphocytes (donor) or 5×10^4 irradiated third-party human lymphocytes (allocontrol) in 200 μl tissue culture medium for 6 days. Cryopreserved baboon lymphocytes were thawed and prepared in 5% baboon serum.

Total complement activity (CH100) was quantitated by an agarose-gel method that measured lysis by test sera of sheep red blood cells (Kallestad, Austin, Texas).[16] Antigen-antibody complexes were detected on stained agarose gels after routine zone electrophoresis.[17]

Chimerism was determined by polymerase chain reaction amplification of the baboon chorionic gonadotropin gene. Primers (CCACCCCATGGTCTCCGTTC and GAACGGGGTGCC-TGCTCC) were selected from the middle exon of the beta subunit of baboon chorionic gonadotrophin such that they did not amplify the corresponding human sequence.[18] The sensitivity and specificity of the method was determined by amplification of baboon DNA serially diluted with human DNA. The quantity of baboon DNA present in the various tissues was estimated from an autoradiogram of a Southern blot of the PCR products after hybridisation with a radiolabelled probe prepared from the baboon gene.

Histopathological studies—Wedge or needle biopsy specimens of the xenograft liver were obtained at operation and on days 12, 24, 55, and 64 post-transplantation. One part was frozen in Optimum Cold Temperature for immunofluorescent studies (IF) and the remainder was fixed in formalin. Anti-baboon antibodies were sought by indirect IF microscopy (tissue crossmatch) with the recipient's serum as the primary antibody, followed by fluorescein-labelled anti-human IgG and IgM as the secondary immunoreactants. Frozen tissue was also studied with direct IF using anti-human IgG, IgA, IgM, C1q, C3, C4, fibrinogen, alpha-2-macroglobulin, and albumin.

Fig 2—Cholangiogram on day 61.

The ducts are much larger than at the time of transplantation and the sharp cut off of a major duct (arrow) was probably because of sludge found at necropsy.

Phenotypic analyses of the cellular infiltrates with routine indirect avidin-biotin complex technique were performed on paraffin-embedded tissues with antibodies directed at T (L60, UCHL-1, OPD4 [CD4]), B (L26, IgG, IgM), and NK (Leu-7) cells, and macrophages and neutrophils (lysozyme). We adopted the same technique to monitor HBV infection (anti-HBsAg and anti-HBcAg) and proliferative activity (anti-proliferating cell nuclear antigen).

Results

Clinical course

After transplantation, our patient, who had lapsed into stage 3 coma preoperatively, woke promptly and was extubated after 17 h. He was eating and walking within 5 days. For the first two postoperative weeks, human albumin 50–75 g/day was given intravenously. Albumin was then administered only to cover losses from dialysis procedures or during plasmapheresis for haemoglobinaemia during the terminal phase of his postoperative course. The liver was 600 g at the time of transplantation and regenerated to a computed tomographic (CT) scan estimated size of 1074 g after one week and 1555 g by day 24. Although he was released from intensive care after one month, he developed several infections (table II) that necessitated treatment with nephrotoxic antibodies. The most disabling of these was mixed CMV and candida oesophagitis and duodenitis, which were suspected to be the cause of recurrent gastrointestinal haemorrhages from days 27–39 and which required 14 units of transfused blood. *Staphylococcus aureus* was cultured from the blood on postoperative days 6, 26, and

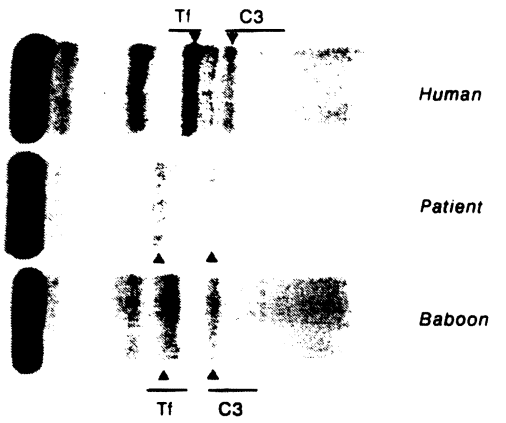

Fig 3—Protein electrophoresis of normal human, baboon donor, and patient serum.

C3, complement; Tf, transferrin.

55; *Enterococcus faecalis* was found cultured on day 55. On day 65, aspergillus was cultured from a tracheal aspirate.

Other complications included renal failure and dialysis dependence beginning on day 21, which probably resulted from multiple drug toxicity (FK 506, amphotericin, ganciclovir, and possibly vancomycin) and a right haemothorax from a liver biopsy on day 24. Despite these difficulties, the patient was afebrile and otherwise well until day 55 when he was readmitted to intensive care after jaundice recurred. Angiography on day 59 showed normal hepatic vascular anatomy; a transhepatic cholangiogram on day 61 was read as normal (fig 2). 1 h after cholangiography, he became hypotensive with rigors; he required intubation. There was evidence of disseminated intravascular coagulation and haemolysis with a fall in platelet count from 115 000 to 29 000 × 10^9/L, an increased free plasma haemoglobin of 87·5 mg/dl (normal < 3·0), undetectable haptoglobin, and a rise in bilirubin from 212 mmol/L to 851·6 mmol/L during the next 48 h.

From days 65 to 70, the patient had 5 plasmaphereses that reduced serum bilirubin. On day 70, while being weaned from the ventilator, there was a sudden loss of higher nervous system function. A CT scan showed a massive subarachnoid haemorrhage and 6 h later he was declared brain dead.

Biochemical studies

The arterial ketone body ratio rose from 0·3 (low) during wound closure to > 1·0 at 3 days and beyond, indicating excellent hepatic energy charge. Tests of liver injury (aminotransferase concentrations) were increased to a maximum of 250 IU/L during the first few days post-transplantation and after the cholangiogram. Bilirubin concentrations were also normal throughout most of the post-operative course (fig 1); however, alkaline phosphatase was increased from the end of the the first week onward.

Prothrombin time and other indices of hepatic synthetic function were similar to those measured preoperatively. The relative hypoalbuminaemia was well tolerated by the patient; he had no evidence of fluid retention at any time. Serum protein electrophoresis in the recipient showed that other protein fractions of hepatic origin promptly reverted to those of the baboon (fig 3). Plasma ammonia concentration was 139 mmol/L preoperatively, and fell to below 50 mmol/L after the first week (normal < 50). Serum uric acid (normally < 30 mmol/L in the baboon) fell from 481·8 mmol/L in our patient into the expected baboon range. A similar but less dramatic transition was seen with serum cholesterol (table I).

Immunological and viral studies

Preoperatively the recipient's serum samples had complement-dependent lymphotoxic antibody activity against 73% to 89% of a 45 cell panel of HLA-typed donors and against lymphocytes from 7 different baboons including the eventual donor. After DTT treatment, the 73–89% panel-reactive antibody before transplantation became 0%, and the patient's positive serum crossmatch with all 7 baboon donors became weak-positive. All serum samples post-transplantation were crossmatch negative after DTT treatment. Daily serum samples tested for donor-specific crossmatch without DTT showed minimum change in titres ranging from 1 in 8 to 1 in 16 throughout the first month post-transplant.

The total white blood cell count preoperatively was 13 × 10^9/L with 6·2 × 10^9/L lymphocytes (28% CD4, 58% CD8; ratio 0·48). After transplantation, the total number of white blood cells remained constant but the number of lymphocytes fell to below 0·6 × 10^9/L. Pretransplantation, he had a normal immune response to several stimuli, including mitogens (PHA and Con A), alloantigens, and other donor-baboon lymphocytes (table III). Postoperatively, our patient's lymphocytes failed to respond to allo or xeno antigens and the only significant proliferation took place with PHA.

The total complement (CH100) fell from normal preoperatively to below the limit of detection (< 21 U/mL) for the first 8 postoperative days during which circulating antigen-antibody complexes were present (table IV). Thereafter, the CH100 ranged from slightly depressed to normal at the same time as complexes were detectable. The results were similar to those observed by Manez et al[16] in successfully treated recipients of lymphocytotoxic-crossmatch-positive allografts. With the return of CH100, C3, which is thought to be synthesised mainly in the liver,[19] was slightly depressed while C4 and C5 were normal. C4 is also thought to be mainly hepatic in origin.[20]

TABLE III—IN-VITRO TESTS OF IMMUNE REACTIVITY

Time (wks)	WBC (× 10^9/L)		Proliferative responses (net cpm)			
	Total	Lymphocytes	PHA	Con A	Xeno MLR	Allo MLR
Pre-operative	13	6·2	90,263	13,371	44,794	74,290
1	10·5	0·5	8,591	436	112	413
2	5·9	0·3	384	90	100	250
4	10·2	0·4	16,788	92	121	62
6	6·9	0·3	1,963	81	59	48
8	5·1	0·6	37,945	4,606	448	156
9	8·4	0·9	1,248	90	120	127

Cpm, counts per minute.

TABLE IV—TOTAL SERUM COMPLEMENT (CH100), COMPLEMENT COMPONENTS (U/mL), AND IMMUNE COMPLEXES (IC)

Days postop	CH100 (>60)	C3 (83–177)	C4 15–45)	C5 (6–20)	IC
1	<21	..	..	..	+
2	<21	..	..	..	+
4	<21	..	..	..	+
6	<21	..	..	..	
8	<21	..	..	..	+
9	<21	..	..	..	−
11	21	..	..	..	−
14	43	..	..	..	−
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	..	..	..	−

Baboon DNA was found in the patient's heart, lung, kidney, and two lymph nodes obtained at necropsy (fig 4). The blood samples collected during life also were positive. In some tissues, baboon and human DNA were found in a ratio of 1 in 1000 whereas in others, such as lymph nodes, the ratio was close to 1 in 10 000 (fig 4).

HIV status did not change significantly after transplantation. Cell dilution method for quantification of HIV-infected cells showed 66 infected cells per million peripheral blood mononuclear cells (PBMC) on days 2 and 17, with 16 infected cells per million PBMC on post-transplant days 31 and 59 (courtesy of Dr Phalguni Gupta, Graduate School of Public Health, University of Pittsburgh).

Pathological studies

Tissue crossmatch of the xenograft before revascularisation with pretransplant recipient serum showed a diffuse non-specific staining pattern with anti-IgG. Anti-IgM revealed faint portal vein staining, focal sinusoidal cell positivity, and distinct nuclear staining consistent with the preformed antibody detected on conventional crossmatch.

The 4 h post-perfusion biopsy revealed Kupffer cell hypertrophy, sinusoidal neutrophilia, and small platelet aggregates in the sinusoids and portal and hepatic veins, without hepatocyte necrosis or inflammatory or necrotising vasculitis. Direct IF showed diffuse sinusoidal fibrinogen (IgG > IgA > IgM) with linear-granular sinusoidal C1q,

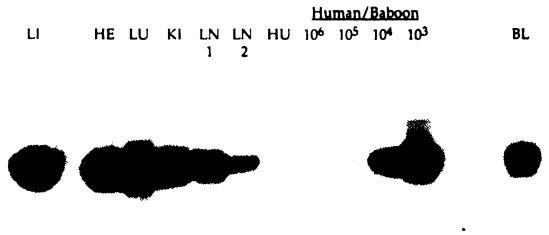

Fig 4—PCR amplification of baboon-specific DNA from recipient tissues.

Lane LI is the PCR product from baboon liver and contains only 1% of PCR reaction to avoid overwhelming the other lanes. HE, heart; LU, lung; KI, kidney. LN1 and LN2, lymph nodes 1 and 2. For semiquantitation, either human (HU) or baboon DNA was serially diluted into human DNA at the indicated ratios and tested, showing a baboon DNA concentration of approximately 0·1% in most specimens. Blood (BL) was obtained 35 days post-transplantation and all other samples were taken at necropsy (day 70).

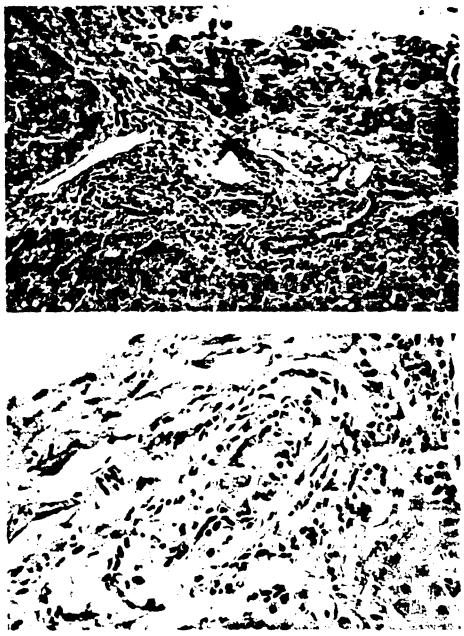

Fig 5—Histopathological studies.

Upper figure: 12 days—portal tract with most severe rejection (×100). Lower figure: 65 days—disruption of epithelium of septal bile duct with no evidence of rejection (×400).

Kupffer cell localisation of C3, and scant C4. Focal mild portal vein and hepatic arterial endothelial IgM deposits also were present. The diagnosis of mild humoral damage was made.

A wedge biopsy taken on day 12 showed Kupffer cell hypertrophy, mild centrilobular hepatocyte swelling, and cholestasis with a mild mononuclear portal and perivenular infiltrate (fig 5 upper). The infiltrate was comprised predominantly of T and NK cells, which were detected inside the basement membrane of a few small septal bile ducts and in the sinusoids. Subset analysis showed that about a third of portal T cells were CD4+, with the remainder presumably CD8+. Inflammatory or necrotising arteritis was not seen. Direct IF findings were qualitatively similar to those of the post-perfusion biopsy, but were less intense. No HBV antigens were detected and a PCNA labeling index in hepatocytes approached 35–40%. Diagnoses of regeneration and of mild cellular rejection (with a small humoral component) were made.

The single needle core of tissues obtained on day 24 was unremarkable except for mild cholestasis and Kupffer cell hypertrophy. Direct IF revealed striking decreases in all immunoglobulins and no detectable complement components. The diagnosis was mild cholestasis. The fourth liver biopsy on day 55 after an increase in total serum bilirubin to 78·7 mmol/L was also unremarkable except for Kupffer cell hypertrophy and a slight increase in sinusoidal mononuclear cells, with little or no portal or perivenular infiltrate and no evidence of inflammatory or necrotising vasculitis. Direct IF examination revealed a slight increase in sinusoidal immunoglobulin deposits with scant but detectable C1q. A diagnosis was made of sinusoidal reactivity with little evidence of rejection; minimum cholestasis was noted.

The fifth (wedge) biopsy was obtained intraoperatively on day 65, 3 days after the transhepatic cholangiogram which had led to a septic crisis. Cholestasis was conspicuous with

several bile infarcts, and focal mural necrosis of occasional septal bile ducts (fig 5 lower). Fewer than half the portal triads contained a mild mononuclear infiltrate, qualitatively similar in phenotypic composition to that seen earlier. More than 30% of bile ducts contained more T cells than NK cells inside the basement membrane, and these were associated with duct epithelial damage. There was no evidence of inflammatory, necrotising, or obliterative arteritis or coagulative vascular necrosis. Direct IF showed a sinusoidal pattern similar to that seen in the post-reperfusion sample, but with no arterial or vein deposits, consistent with the lack of vasculitis on light microscopy. Stains for HBV antigens were negative. A diagnosis of mild rejection, cholestasis, and bile infarcts, consistent with large duct obstruction, was made.

Endoscopic biopsy specimens of either the oesophagus, stomach, and/or duodenum were obtained on days 29, 30, 39, 43, and 55 because of complaints of anorexia and abdominal pain. Candida and severe cytomegalovirus oesophagitis were diagnosed on days 29 and 39, respectively.

Necropsy

The cause of death was diffuse subarachnoid haemorrhage and left uncul brainstem herniation, secondary to invasive aspergillosis in multiple sites in the left brain; haemorrhagic aspergillus lesions were also found in the left and right lungs, and left kidney. Other findings in the kidney were mild arterial nephrosclerosis, glomerular lobular accentuation, mesangial expansion and hypercellularity, and acute tubular necrosis. There was residual CMV oesophagitis and gastritis.

The hepatic arterial, portal venous, and vena caval anastomoses were patent. The biliary anastomosis was intact and patent, but the bileduct mucosa was dusky. Biliary sludge occupied the entire intrahepatic biliary tree, from the first-order branches through the extrahepatic ducts. Chemical studies of the material were characteristic of pigment sludge that, on histopathological examination, consisted of a large component (80%) of cellular debris. This finding was similar to that seen in past allografts with biliary stasis or obstruction. Multiple bile infarcts were present throughout the liver. There was no gross or histopathological evidence of inflammatory or obliterative arteriopathy of the deep hilar, peripheral, and peri-anastomotic graft arteries. There was no significant portal infiltrate in any of the multiple samples.

Discussion

We have reported previously that the incidence and management of liver allograft rejection were not substantially different in HIV-positive liver recipients than in those testing HIV negative.[21] Because our patient was immunocompetent before transplantation, the immunosuppression postoperatively was thought to be iatrogenic, not due to HIV. We believe that the four drug immunosuppressive regimen of FK 506, prednisone, PGE, and cyclophosphamide was effective in preventing both cellular rejection and the occlusive endotheliolitis attributed to antibody-mediated rejection in previously described baboon-to-human xenografts.[4,5]

The fatal outcome followed unrecognised biliary stasis with consequent diffuse damage of intrahepatic ducts, sludge formation, and bacterial infection. The raised alkaline phosphatase and jaundice were ascribed to rejection and treated as such despite the lack of histopathological evidence for this diagnosis. Similar technical and management difficulties of the biliary system, which ultimately proved correctable impeded early development of liver allotransplantation more than any other single factor.[22] It is likely that the biliary stasis found in our hepatic xenograft is preventable by stenting the biliary anastomosis with an exteriorised catheter that can also be used for cholangiography or irrigation postoperatively. However, we cannot exclude duct-specific immunologic injury or even chemical injury from a drug such as cyclophosphamide.

Our acknowledgement that failure in this case was probably technical permits an encouraging interpretation of other observations in this patient. The most important modification of immunosuppression must be restriction of cyclophosphamide to the perioperative period, as is necessary for prevention of antibody-mediated rejection of hamster organs transplanted to rats.[6,7] Because of the fear of organ rejection in our patient, this drug was continued for nearly two months with probable over-immunosuppression. However, the preformed lymphocytotoxic IgM antibodies present preoperatively in our patient's serum did not increase after transplantation, and no circulating cytotoxic IgG antibodies were ever measurable postoperatively. Diffuse IgM and IgG antibodies demonstrated in the xenograft biopsy sample by immunofluorescence at 12 days had largely disappeared by 24 days.

The liver xenograft is thought to have immunological advantages compared with other potentially transplantable organs, including greater resistance to humoral rejection and an unusual ability to take part in the induction of its own acceptance.[23] These advantages are explained by a high density of potentially migratory leukocytes of bone-marrow origin, including Kupffer cells, which are crucial to the development of systemic recipient chimerism.[24,25] Circulating baboon DNA was demonstrated by polymerase chain reaction after 35 days in our xenograft recipient, and at necropsy chimerism was confirmed in all tissues and organs tested. This finding after allo or xeno transplantation is construed as the first step toward chronic acceptance and ultimately donor-specific tolerance.[24]

An additional question largely answered by this single case is whether production by a liver xenograft of donor phenotype proteins and other synthetic products would result in a lethal incompatibility of metabolism in the recipient. The fall in serum uric acid and cholesterol concentration postoperatively to the low values that are normal for the baboon was an especially dramatic demonstration of the recreation by the xenograft of its own chemical environment and with no apparent adverse effects. As expected, the serum protein pattern of the recipient rapidly approached that of the baboon, including proteins involved in immune reactions or blood coagulation. The low serum albumin produced by the xenograft was well tolerated. These observations and those in rat recipients of hamster livers[26] suggest that donor-specific products of hepatic synthesis will not preclude liver xenotransplantation from a concordant species donor. It was also noteworthy that liver regeneration of the originally small xenograft took place at a similar time course described for the human liver.

Death at 70 days was too soon to allow a conclusion that the transplanted baboon liver could successfully resist infection with HBV. However, there was no evidence of the HBsAg or HBcAg in the transplant at necropsy. Infection of allografts with HBV has been recorded frequently during this time.[28] Since the baboon cannot be easily infected with HIV (if at all),[29] longer observation might have provided

data about HIV status of the chimeric baboon lymphodendritic cells of monocyte/macrophage lineage, which are thought to account for the post-transplant chimerism that is invariably found after successful hepatic allotransplantation. Evidence of chimerism was widespread in the xenograft recipient's organs. The crucial question will be whether HIV-resistant chimeric xenogeneic cells would have a survival advantage over infected autologous cells of the same lineage, and whether this could ultimately influence the course of HIV infection favourably. An additional question raised by the recent observation that FK 506 and cyclosporin interfere with HIV production and selectively inhibit growth of infected cells[30] is whether chronic immunosuppression with these drugs could have an antiviral effect. The long survival of several HIV-infected liver recipients is compatible with this possibility.[31]

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REFERENCES

1. Hitchcock CR, Kiser JC, Telander RL, Seljeskog EL. Baboon renal grafts. *JAMA* 1964; **189:** 158–61.
2. Starzl TE, Marchioro TL, Peters GN, et al. Renal heterotransplantation from baboon to man: experience with 6 cases. *Transplantation* 1964; **2:** 752–76.
3. Barnard CN, Wolpowitz A, Losman JG. Heterotopic cardiac transplantation with a xenograft for assistance of the left heart in cardiogenic shock after cardiopulmonary bypass. *S Afr Med J* 1977; **52:** 1035–39.
4. Bailey LL, Nehlsen-Cannarella SL, Concepcion W, Jolley WB. Baboon-to-human cardiac xenotransplantation in a neonate. *JAMA* 1985; **254:** 3321–29.
5. Porter KA. Pathological changes in transplanted kidneys. In: Starzl TE. Experience in renal transplantation. Philadelphia: Saunders, 1964: 284–98.
6. Hasan RIR, Bogaerde van den J, Forty J, Wright L, Wallwork J, White DJG. Prolonged survival of hamster to rat xenografts with cyclophosphamide therapy. *Transplant Proc* 1992; **24:** 517–18.
7. Murase N, Starzl TE, Demetris AJ, et al. Hamster to rat heart and liver xenotransplantation with FK506 plus antiproliferative drugs. *Transplantation* (in press).
8. Hasan RI, Sriwatanawongsa V, Wallwork J, White DJG. Consistent prolonged "concordant" survival of hamster-to-rat cardiac xenografts by inhibition of anti-species antibody production with methotrexate. *Transplant Proc* (in press).
9. Moran M, Mozes MF, Maddux MS, et al. Prevention of acute graft rejection by the prostaglandin E_1 analogue misoprostol in renal-transplant recipients treated with cyclosporine and predonisone. *N Engl J Med* 1990; **322:** 1183–88.
10. Takaya S, Iwaki Y, Starzl TE. Liver transplantation in positive cytotoxic crossmatch cases using FK506, high dose steroids and prostaglandin E1. *Transplantation* 1992; **54:** 927–30.
11. Cano MJ, Desco M, Garcia-Barreno P. Normal biochemistry values in baboons *(papio C Cinocephalus)*. *Comp Biochem Physiol* 1990; **96B:** 647–49.
12. Starzl TE, Demetris AJ. Liver transplantation: a 31-year perspective. Chicago: Year Book Medical Publishers, 1990: 18–36.
13. Takaya S, Bronsther O, Abu-Elmagd K, Fung JJ, Starzl TE. The use of prostaglandin E1 in crossmatch negative liver transplant recipients treated with FK 506. *Transplant Proc* (in press).
14. Asonuma K, Takaya S, Selby R, et al. Clinical significance of the arterial ketone body ratio as an early indicator of graft viability in human liver transplantation. *Transplantation* 1991; **51:** 164–71.
15. Iwaki Y, Lau M, Terasaki PI. Successful transplant across T-warm IgM positive crossmatches. *Clin Transplant* 1988; **2:** 81–94.
16. Manez R, Kobayashi M, Takaya S, et al. Humoral rejection associated with antidonor lymphocytoxic antibodies following liver transplantation. *Transplant Proc* (in press).
17. Kelly RH, Scholl MA, Harvey VS, Devenyi AG. Qualitative test for circulating immune complex by use of zone electrophoresis on agarose. *Clin Chem* 1980; **26:** 396–402.
18. Crawford RJ, Tregear CW, Niall HD. The nucleotide sequences of baboon chorionic gonadotropin beta-subunit genes have diverged from the human. *Gene* 1986; **46:** 161–69.
19. Alper CA, Johnson AM, Birtch AG, Moore FD. Human C3: evidence for the liver as the primary site of synthesis. *Science* 1969; **163:** 286–88.
20. Wolpl A, Robin-Winn M, Pichlmayr R, Goldman SF. Fourth component of complement (C4) polymorphism in human orthotopic liver transplantation. *Transplantation* 1985; **40:** 154–57.
21. Tzakis AG, Cooper MH, Dummer JS, Ragni M, Ward JW, Starzl TE. Transplantation in HIV + patients. *Transplantation* 1990; **49:** 354–58.
22. Starzl TE, Putnam CW, Hansbrough JF, Porter KA, Reid HAS. Biliary complications after liver transplantation: with special reference to the biliary cast syndrome and techniques of secondary duct repair. *Surgery* 1977; **81:** 212–21.
23. Valdivia L, Demetris AJ, Fung JJ, Celli S, Murase N, Starzl TE. Successful hamster to rat liver xenotransplantation under FK 506 immunosuppression induces unresponsiveness to hamster heart and skin. *Transplantation* (in press).
24. Starzl TE, Demetris AJ, Murase N, Ildstad S, Ricordi C, Trucco M. Cell migration, chimerism, and graft acceptance. *Lancet* 1992; **339:** 1579–82.
25. Starzl TE, Demetris AJ, Trucco M, et al. Systemic chimerism in human female recipients of male livers. *Lancet* 1992; **339:** 1579–82.
26. Valdivia L, Lewis JH, Celli S, et al. Hamster coagulation protein activities in hamster-liver transplanted rats. *Transplantation* (in press).
27. Francavilla A, Panella C, Palimeno L, et al. Hormonal and enzymatic parameters of hepatic regeneration in patients undergoing major liver resections. *Hepatology* 1990; **12:** 1134–38.
28. Todo S, Demetris AJ, Van Thiel D, Fung JJ, Starzl TE. Orthotopic liver transplantation for patients with hepatitis B virus (HBV) related liver disease. *Hepatology* 1991; **13:** 619–26.
29. Essex M. Origin of AIDS. In: De Vita VT, Hellman S, Rosenberg SA (eds). AIDS etiology, diagnosis, treatment, and prevention. Philadelphia: Lippincott, 1992: 3–11.
30. Karpas A, Lowdell M, Jacobbon AK, Hill F. Inhibition of human immunodeficiency virus and growth of infected T cells by the immunosuppressive drugs cyclosporin A and FK 506. *Proc Natl Acad Sci (USA)* 1992; **89:** 8351–55.
31. Jacobson SK, Calne RY, Wreghitt TG. Outcome of HIV infection in transplant patient on cyclosporin. *Lancet* 1991; **337:** 794.

From The Lancet

Ocean travel

Life at the end of the nineteenth century is so eager and full of struggle, competition is so keen, the reward of successful effort so great, that most of us in every station neglect those precautions and habits upon which continued health chiefly depends. Prolonged and anxious intellectual labour, irregular hours, warm and ill-ventilated rooms, insufficient sleep and exercise, strain the nervous system, beget indigestion and innumerable physical evils. The physiological and prophylactic remedies of out-door exercise, rest, diet, rational amusement, which do so much to keep us in health, are not so generally utilised as their inestimable value deserves. Accordingly we approach the summer season jaded, worn, with nerves unstrung, appetite feeble, digestion faulty, sleep restless and insufficient. Rest is urgently demanded; the brain needs to lie fallow for a time that it may recuperate after its strenuous energy. Among the many means to which we may resort for restoration of physical and intellectual force I would unhesitatingly assign a high, perhaps the highest, place to ocean travel .... Life at sea is sufficiently full of incident to avoid the reproach of dullness. One meets with old friends or forms new acquaintances and a pleasant, leisurely intercourse permits the days to glide by without weariness. The sea air, always in circulation and charged with ozone, invigorates the exhausted system. Movement is not restrained. We feel the ship beneath us forging its way through the water, the deck affords us an ample promenade, the heaving surface of the ocean a picture full of charm. The pure salt breeze fans our cheeks, the porpoise or the shark claims a share of our attention, and as we traverse the ocean highway we meet other steamers sending up their clouds of smoke or sailing vessels with canvas spread to catch the wind.

(July 23, 1892)