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Xenotransplantation

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58 Baboon Liver Xenotransplantation in Humans: Clinical Experience and Principles Learned

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Introduction

The significant advances achieved in the field of liver transplantation have led to an increased demand for organs, and created a wide gap between organ availability and supply [1, 2]. As of 3 January 1996, there were 44 025 patients on the United Network for Organ Sharing (UNOS) waiting list (the agency that coordinates organ allocation in the United States), up from 13 115 on 31 December 1987, an increase of 335 %. Of these, 5715 awaited liver transplantation, up from 449 in 1987 (12-fold increase). The supply of organ donors, on the other hand, underwent a marginal increase between 1988 and 1990 (from 4085 to 4514), and has remained relatively stable (4531 in 1991, 4521 in 1992, 4849 in 1993, and 4891 in 1994) thereafter. A wider availability of organs for transplantation would allow an expansion [3, 4] rather than a contraction of the indications for transplantation, and at the same time a relaxation of the patient selection criteria [5–7]. All these facts clearly justify the renewed interest in xenotransplantation observed in the last decade [8, 9].

The first three attempts at whole organ xenotransplantation were made in France and Germany between January and April 1906, using a pig, a goat, and a macaque as kidney donors [10, 11]. None of these kidneys functioned because of almost immediate vascular thrombosis, and the human recipients died in less than 3 days. In a further attempt in 1923 by Neuhof [12], a lamb was used as a kidney donor, and the patient died 9 days later. On 16 February 1963, Hitchcock transplanted the kidney of a baboon into a 65-year-old woman. The organ functioned for 4 days before its artery clotted [13]. A few months later, on 8 October 1963, Reemtsma of Tulane University used a rhesus monkey as kidney donor for a human recipient, who survived 12 days. Reemtsma tried again with a series of six consecutive chimpanzee kidney grafts [14], one of which functioned for 270 days.

In December 1963 and January 1964, six patients received baboon kidneys at the University of Colorado in Denver [15]. Each of these kidneys functioned immediately and sustained a dialysis-free life for 10–60 days. The patients were treated with high doses of azathioprine and prednisone; four of them died of sepsis, while rejection was mainly responsible for the other two deaths. However, the pathology of the rejection was not qualitatively different from that observed in allografts [16].

In fact, although the pathology of the rejection process was not well understood during the early era of xenotransplantation, the descriptions of the rejected xenografted kidneys, from both baboons and chimpanzees, are consistent with

the pathophysiology of xenograft rejection as we describe it today. Lymphocytotoxic antibodies as a cause for antibody-mediated rejection of allografts were actually not recognized until 1965 [17]. In the Denver baboon-to-human kidney xenotransplant series mentioned above, heterospecific antibodies could be detected bound to the kidney xenografts [15, 16]. Summarizing that experience, Dr. Kendrick Porter concluded:

> In the resulting [heterograft] rejection process, cellular infiltration and peritubular capillary destruction are prominent early pathologic features, but by nine days the vasculonecrotic element is marked. There is circumstantial evidence to suggest that, whereas the peritubular capillary damage is mediated by cell-bound antibody, the fibrinoid necrotic vascular lesions are caused by circulating antibody.

Porter also noted that the rejected xenografts showed variability in the histology, from total infarction, cellular infiltrates, interstitial edema and tubular necrosis, to only intimal hypertrophy. The antibody component of rejection has been the central issue of xenotransplantation since that time.

A pig kidney and a pig heart, transplanted in the 1960s by Kuss [18] and Ross [19], respectively, were hyperacutely rejected in a matter of minutes, demonstrating that the pig was not, and will not be, an easy donor for a human recipient.

In 1968, the guidelines for defining brain death were published in the *Journal of the American Medical Association* [20]. Almost overnight, the availability of brain-dead, heart-beating cadaver donors eliminated the need to continue the quest for nonhuman donor organs. Widespread access to dialysis and United States government financing of the end-stage renal disease program allowed patients with kidney failure to live and wait for kidney transplantation, whereas previously kidney transplantation was the only alternative to death. Interest in xenotransplantation of kidneys gave way to pretransplant management with dialysis, and more timely transplantation with optimally functioning human kidney allografts. However, in the following two decades, the success achieved with allotransplantation triggered a new organ shortage crisis, highlighted by the increasing number of patients dying while waiting for a human organ. This is particularly disturbing in certain candidate populations, such as neonates and small children, where donor scarcity is even more severe. Such was the rationale for the only attempt at human xenotransplantation in the cyclosporine era, when the shortage of neonatal hearts induced Bailey and coworkers [21] to use a baboon heart in a 2.2-kg neonate recipient in 1984. In spite of the cyclosporine, steroid, azathioprine, and antithymocyte globulin treatment, immunopathological events similar to the ones observed by Porter [16] 20 years before brought the baboon heart to irreversible failure within 3 weeks. No further attempts were made for almost a decade, until three human liver xenotransplantations, using two baboons and one pig, were performed in 1992 and 1993 [22–24]. The description of the two baboon-to-human liver xenotransplantations, and the principles learned with this experience, are the subject of this chapter.

Work in the Laboratory and the Consensus Process

In 1969, Sir Peter Medawar [25] stated:

> A new solution is therefore called for: the use of heterografts – that is to say, of grafts transplanted from lower animals into man. Of the use of heterografts I can say only this: that in the laboratory we are achieving greater success with grafts *between* species today than we achieved with grafts *within* 15 years ago. We shall solve the problem by using heterografts one day if we try hard enough, and maybe in less than 15 years.

However, the laboratory work performed at different institutions in the following 15–20 years did not bring results that could encourage further clinical trials.

In May 1992, a study performed in Pittsburgh by Murase et al. [26], using a hamster-to-rat xenotransplant model, was discussed at the meeting of the American Society of Transplant Surgeons in Chicago. Murase's work clearly showed that indefinite survival under tacrolimus (formerly FK506) was routinely achievable if it was combined, for the first two post-transplant weeks, with either of two "antiproliferative" drugs: mycophenolate mofetil (an inhibitor of purine synthesis) or brequinar sodium (an inhibitor of pyrimidine synthesis). The use of cyclophosphamide, an alkylating agent with considerable B cell specificity [27, 28], allowed similar consistent chronic survival after either heart or liver xenotransplantation. Particularly significant was the fact that a single large dose of cyclophosphamide, given 10 days before the xenotransplant, allowed success in almost 100% of the animals with only daily administration of tacrolimus. This work, together with the previous experience with cyclophosphamide [29, 30] as an effective drug in clinical transplantation, justified its use in a clinical xenograft trial.

Pittsburgh Clinical Trial

Planning

Starting from this premise, in November 1991 the Pittsburgh Transplantation Institute notified the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health (J. Hoofnagle and P. Gordon), the Food and Drug Administration (R. Lieberman and G. Burke), and the Secretary of the Department of Health and Human Services (L. Sullivan) of the intention to proceed with the clinical liver xenotransplant project. Eight months were then needed to present the scientific documentation to the appropriate government agencies in the United States, the Institutional Review Board and the Ethics Committee of the University of Pittsburgh Medical Center, and members of the United States Congress with special interest in health care problems [31]. Also, in March 1992, a committee formed by six eminent European and American surgeons, coordinated by Keith Reemtsma of Columbia University, was brought together to hear the opinions of other experts before sanctioning the first clinical baboon liver xenotransplant.

After making several modifications to the initial protocol, on the basis of suggestions made by the various experts consulted, the first xenotransplant was performed on 28 June 1992, and the second on 10 January 1993. During the long interval between the first and the second, and despite authorization to perform four consecutive liver xenotransplants [32], it was chosen to bring together the same group of experts previously consulted, this time at the New York Academy of Medicine, so that they could analyze the results obtained in the first xenotransplant. On this occasion, the Pittsburgh Transplantation Institute was advised to continue the clinical trial.

The protocol for this human trial (approved by the Institutional Review Board of the University of Pittsburgh Medical Center [32] allowed baboon-to-human liver transplantation for the treatment of end-stage cirrhosis related to chronic active hepatitis B virus. In many areas of the world, infection with hepatitis B virus is endemic. Over 250 million people are affected by this disease worldwide [33], and it is also the most common cause of advanced liver disease and hepatocellular carcinoma. Recurrence of infection after liver transplantation is high in this patient population, especially in the subset with positive viral replication markers (e.g., hepatitis B "e" antigen and/or hepatitis B DNA positivity) [34, 35]. In many institutions this disease is considered a contraindication for liver allotransplantation. Because the baboon liver is thought to be resistant to the development of chronic active hepatitis B [22], the principal benefit to the patients enrolled in this trial was the possibility that the xenotransplanted liver would not be reinfected by the viral disease. The transplant was therefore planned as a permanent replacement for the failing human liver.

Choice of Donor Species

Nonhuman primates, by sharing many physiologic and genetic characteristics with humans, seemed to qualify as donors for this attempt. Primates are comprised of two suborders, *Prosimii* and *Anthropoidea*. Prosimian primates resemble squirrels or rats more than true monkeys. The *Anthropoidea* suborder can be further subdivided into families: New World monkeys, Old World monkeys, lesser apes, great apes, and man. From an investigational standpoint, the most frequently used species are the Old World monkeys. This family includes rhesus monkeys (*Macaca mulata*), cynomologus monkeys (*Macaca fascicularis*), and baboons (*Papio cynocephalus*, *Papio sphinx*, *Papio gelada*, and *Papio hamadryas*) [36]. Great apes include the chimpanzee (*Pan troglodytes*) and gorilla (*Gorilla gorilla*), but are not used in great numbers for research because of their endangered classification, although their genome and their size approximate those of humans more than lesser apes.

The liver anatomy in all primates is similar, with a right and left lateral lobe placed dorsally and a single large ventral central lobe. The liver of the Macaca and Papio is notable for lobation, with four identifiable lobes. In the higher order of primates, the central lobe fuses with the right and left lateral lobes. The quadrate lobe is much narrower in non-human primates than in man, and the caudate lobe may fully encircle the inferior vena cava. The ligamentous

attachments are similar to those described in man. The portal venous system is essentially identical in the higher order of primates. The hepatic venous drainage is similar to man, with small short hepatic venous tributaries draining the right and central lobes, with two large hepatic venous branches, one right and one left. In all primates, the gallbladder lies closely attached to the right or central lobe. The gallbladder arterial supply is usually from a branch of the right hepatic artery. The common bile duct empties into the duodenum.

The chimpanzee is most likely the best donor in biological terms, due to the very small genetic differences between this species and humans. However, their endangered status prevents their widespread use for scientific purposes. In the United States, only 25–50 chimpanzees may be used annually in biomedical research, including those used in acquired immunodeficiency syndrome (AIDS) research [37], and it is estimated that only 70 chimpanzees would be available worldwide as organ donors each year [38]. Therefore, it was decided that the donor in the Pittsburgh clinical trial would be the baboon, *Papio cynocephalus.*

Donor Baboon Selection Criteria

The baboons used as donors came from the Southwest Foundation for Research and Education, San Antonio, Texas, the same institution that supplied the baboons used in the previous kidney xenotransplant trial [15]. All the baboons used during donor selection were born in the United States [36].

Baboons have group A, B, and AB antigens weakly expressed in all cells, with group O baboons being extremely rare [39–41]. However, ABO incompatibility did not affect the results of previous clinical xenotransplant trial [15, 42]. An ABO match is desirable in a baboon-to-human xenotransplant, but its absence does not constitute an absolute contraindication. Both of our human recipients in this trial, blood group A and B, respectively, received livers from compatible histo-blood group donors.

In addition to histo-blood group, donor selection criteria included a lymphocytotoxic crossmatch, as well as a complete biochemical, viral and bacteriological evaluation of the animals [22]. In particular, infectious disease screening was performed at the Virus Reference Laboratory of the Southwest Foundation for Research and Education, San Antonio, Texas. All potential donors were screened for retroviruses (simian T lymphocytic virus, STLV; human T lymphocytic virus, HTLV; simian immunodeficiency virus, SIV; SRV-1; SRV-2; SRV-5; human immunodeficiency virus-1, HIV-1; HIV-2; foamy virus), herpesviruses (SA-8; herpes simplex virus, HSV; B virus; rCMV, cytomegalovirus; human CMV, hCMV; Epstein-Barr virus, EBV; varicella-zoster virus, VZV), and hepatitis A (HAV), B (HBV), and C (HCV) viruses. In addition, the baboons were examined to exclude tuberculosis and toxoplasmosis, and routine cultures of blood and feces were performed [43].

Baboons are physically smaller than humans. The maximum weight of an adult male baboon is about 30–35 kg. However, a liver from a smaller donor can rapidly grow to accommodate the size of a larger recipient [44]. A number of suitable donors were selected and quarantined before the xenotransplant.

Case Reports

Donor Procedure

The donor operation was performed using the traditional technique described by our group [45, 46]. The operations on the donor and the recipient were performed simultaneously, in two different operating rooms. Cold ischemia times were 80 min in the first case and 231 min in the second. University of Wisconsin solution was used to preserve the organs, as is done in our routine clinical practice.

Recipient Selection

The first patient was a 35-year-old white male, who had undergone a splenectomy in 1989 following a motor vehicle accident. The patient was found to be HIV positive, but his CD4 lymphocyte count was normal, as were in vitro mitogen responses. The second patient was a 62-year-old white male with no previous abdominal surgery. Both patients had end-stage liver disease secondary to chronic active hepatitis B. In both patients, the principal complications of the liver disease were poorly controlled edema, fatigue, ascites, encephalopathy, and gastrointestinal bleeding.

Operative Technique

The liver xenotransplants were performed using a modification of the standard method [47], described 33 years ago [48], and employing a venovenous bypass [49]. The difference in diameter between the vessels of donor and recipient, and the small size of the donor's liver (600 cm^3 and 450 cm^3 in the first and second cases, respectively) made it necessary to use the piggy-back method [50]. In both cases the right suprahepatic vein of the recipient was closed with a running suture, while the left and middle suprahepatic veins were used to perform the suprahepatic vena cava anastomosis. In the first patient, given the considerable size discrepancy between the recipient and donor portal veins, the latter was anastomosed end-to-end onto the recipient's left portal branch, and the right branch was closed with a running suture. In the second case, a smaller discrepancy made it possible to perform a normal end-to-end portal anastomosis.

The donor's celiac axis was anastomosed end-to-end onto the recipient's common hepatic artery in the first case, and end-to-side onto the supraceliac recipient aorta (using a donor carotid artery interposition graft) in the second. In both cases, the liver reperfused uniformly and produced bile on the operating table. The biliary anastomosis was done using a choledochojejunostomy on a Roux loop. In the second case, a small (3.5F) diameter catheter was placed into the biliary anastomosis and brought out through the abdominal wall (Fig. 1), in order to gain direct access to the bile duct to study its anatomy and take bile samples during the postoperative period.

Fig. 1. Second liver xenograft (baboon-to-human) recipient. Cholangiogram performed on postoperative day 18 by injection through a percutaneous biliary catheter. This catheter was placed during the xenotransplant operation (10 January 1993) across the choledochojejunostomy and allowed study of the biliary anatomy as well as daily collection of bile samples after the xenotransplantation. (From [96])

Immunosuppressive Therapy

The immunosuppressive protocol comprised four drugs: cyclophosphamide, tacrolimus, methylprednisolone, and prostaglandin E_1. Cyclophosphamide was begun 2 days prior to the transplant, and administered for a total of 56 days in the first case and 10 days in the second, at a dosage varying between 0.07 and 10.6 mg/kg per day. Tacrolimus, steroids, and prostaglandin were started on the day of the transplant, using the same protocol we follow in clinical liver allotransplantation. Detailed descriptions of the immunosuppressive drug dosing and blood levels obtained are shown in Figs 2 and 3.

Postoperative Course

The first patient was extubated 17 h after the operation, he was placed on an oral diet on the fifth post-transplant day, and lived for 70 days. He spent most of this time in a regular hospital ward and had an almost normal plasma bilirubin level for the majority of the 70 days that he survived. The second patient, who was much older, never regained consciousness, remained icteric, and survived for only 26 days. Figures 2 and 3 illustrate their postoperative courses. The alkaline phosphatase was always very high in the first patient [51] (Fig. 2), while in the

second patient it was not as conspicuously high, although it was always above normal limits.

The first patient underwent five liver biopsies, while the second had seven. Only the biopsy obtained from the first patient on the 12th postoperative day

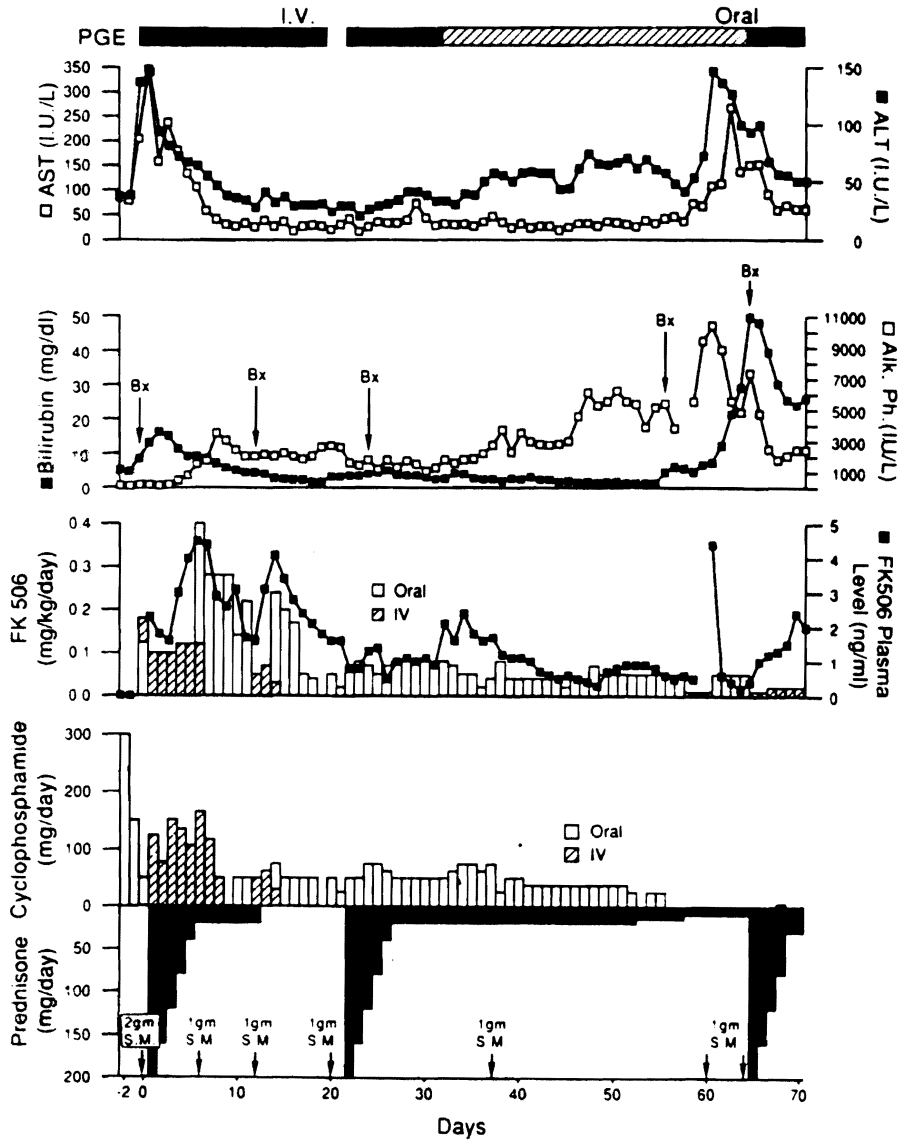

Fig. 2. Clinical course of the first liver xenograft (baboon-to-human) recipient (28 June 1992). *S.M.*, Solumedrol (methylprednisolone); *PGE*, prostaglandin E_1; *Bx*, biopsy; *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *Alk. Ph.*, alkaline phosphatase; *FK506*, tacrolimus. (From [22])

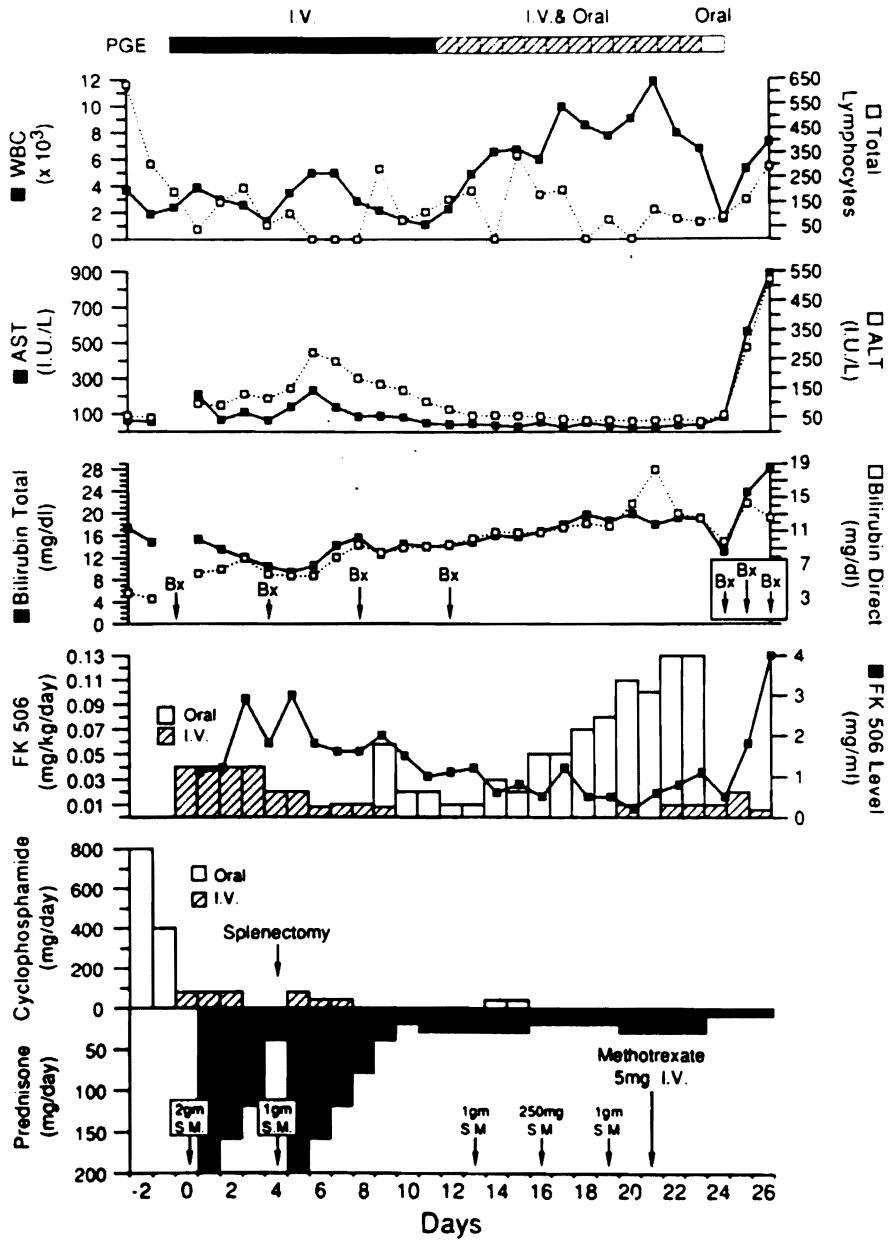

Fig. 3. Clinical course of the second liver xenograft (baboon-to-human) recipient (10 January 1993). *S.M.*, Solumedrol (methylprednisolone); *PGE*, prostaglandin E_1; *Bx*, biopsy; *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *WBC*, white blood cells; *FK506*, tacrolimus. (From [52])

showed signs of mild focal cellular rejection, while no evidence of acute cellular rejection (according to the criteria routinely used in liver allotransplantation), was detected in any of the other biopsies from either patient [22, 23, 31, 52]. However, direct immunofluorescence demonstrated the presence of endothelial deposits of immunoglobulins (IgG>IgA>IgM) and complement (particularly Clq) in both cases [53]. No evidence of HBV reinfection was detectable by immunoperoxidase staining in the liver tissue at any time. Since the period of follow-up was short, no definitive conclusions can be reached regarding the possibility of long-term resistance of the baboon liver to hepatitis B recurrence. However, the most sensitive assay using polymerase chain reaction (PCR) could not detect hepatitis B DNA in the transplanted livers [54].

Macroscopically, considerable hepatic regeneration was noted in both cases, with a significant increase in the volume of the baboon organs. Computed tomography (CT) was used to calculate the volume of the transplanted livers [55]. Both livers showed an extremely rapid growth, as normally occurs when a human liver is transplanted into a recipient with a larger abdomen than the donor [44]. Figures 4 and 5 show the CT performed on the two recipients on the 26th and 14th postoperative days, respectively. The first patient's liver grew from an initial volume of 600 cm^3 to 1555 cm^3 in 26 days. The second patient's liver grew from an initial volume of 450 cm^3 to 1741 cm3 in 14 days. An angiogram performed on postoperative day 59 in the first patient showed that the vasculature of the transplanted xenograft had scaled up appropriately, i.e., the architectural relationships were preserved as the liver increased in volume.

Papio cynocephalus normally produces elevated levels of factor VII and low levels of factors IX and XI, as compared to humans. Coagulation profiles were measured in both recipients preoperatively and several times postoperatively. Our results [56] showed that the baboon's coagulation pattern was acquired by

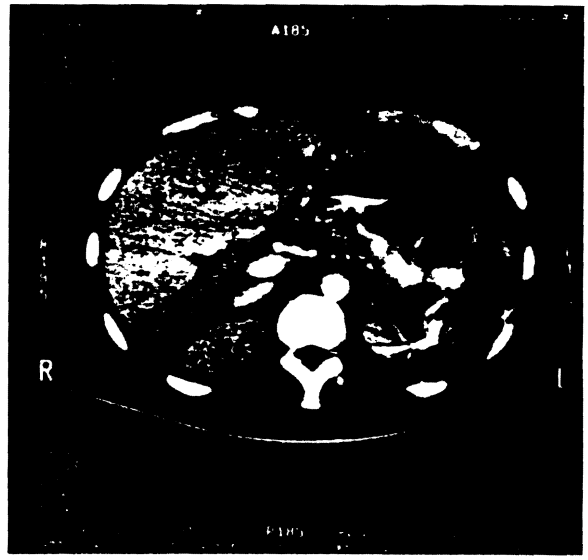

Fig. 4. First liver xenograft (baboon-to-human) recipient. Abdominal computed tomography (CT) on postoperative day 26. The liver volume has increased to 1555 cm^3, from an initial value of 600 cm^3. (From [96])

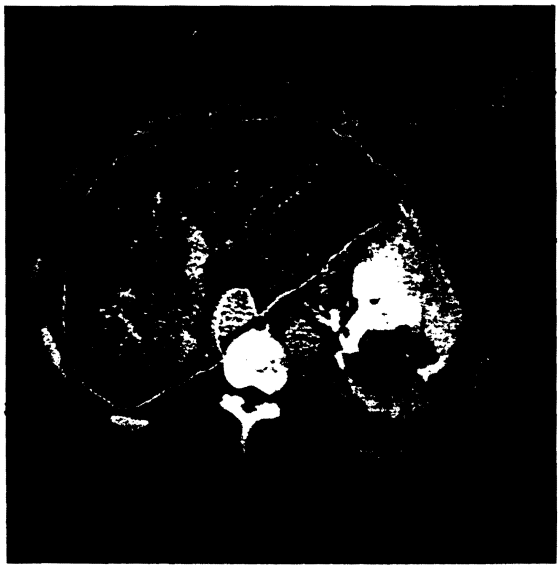

Fig. 5. Second liver xenograft (baboon-to-human) recipient. Abdominal computed tomography (CT) on postoperative day 14. The liver volume has increased to 1741 cm^3, from an initial value of 450 cm^3. (From [96])

the patients after liver xenografting, but this did not affect their clotting ability. No adverse effects of the presence of baboon proteins, such as immune-mediated kidney injury, could be detected at any time.

In both patients there was evidence of an adequately functioning liver mass, such as: (a) normalization of coagulation with normal prothrombin time, (b) correction of hyperammonemia, (c) normal arterial ketone body ratio (a reflection of hepatic energy stores) [57], and (d) clearance of serum lactate. However, both patients suffered from hypoalbuminemia and needed to receive frequent albumin infusion. The baboon liver did impact on the protein profile of both patients. Liver-specific proteins could be shown to be of baboon origin by serum protein electrophoresis (Fig. 6). Total complement levels were depleted for 1–2 weeks after liver xenotransplantation, similar to that reported in liver allotransplantation across a positive lymphocytotoxic crossmatch [9, 22, 23, 52, 53, 58].

The first patient went into renal failure on postoperative day 21, while the second patient became anuric immediately after surgery. Both patients suffered a number of infectious complications. Many of the organisms involved were typical of the agents seen after allotransplantation, e.g., *Staphylococcus*, *Candida*, CMV, *Enterococcus*, and *Aspergillus*. In the first patient, both the recipient and donor were CMV positive. Attempts to determine the origin of the subsequent CMV infection to either human or baboon origin failed because of inability to culture the CMV from clinical samples in sufficient quantity to subtype. However, the susceptibility of both baboon and human CMV to ganciclovir makes the origin of this virus of lesser practical importance.

The causes of death in both patients were multifactorial. In the first, a rise in both the alkaline phosphatase and total bilirubin prompted a percutaneous transhepatic cholangiogram on the 61st post-transplant day. Within 1 h after the pro-

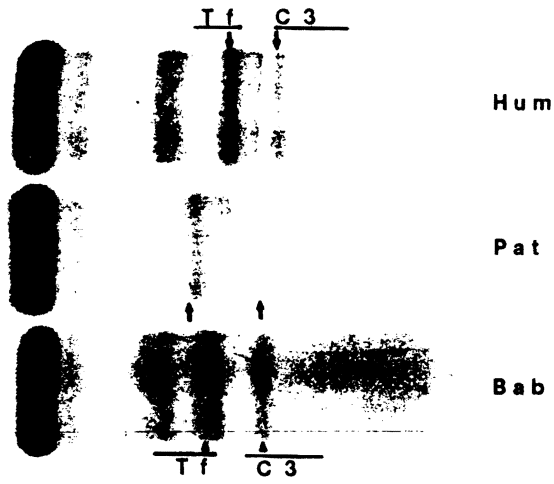

Fig. 6. First liver xenograft (baboon-to-human) recipient. Protein electrophoresis of normal human (*Hum*), baboon donor (*Bab*), and patient serum (*Pat*). *C3*, complement; *Tf*, transferrin. (From [22])

cedure, the patient became hypotensive, febrile, and coagulopathic. He was intubated, stabilized, and his clinical condition improved significantly. A positive culture for *Aspergillus flavus* was noted, and amphotericin treatment was started. On the last day of his life (post-transplant day 70), the patient was hemodynamically stable and was being weaned from the ventilator. Early in the afternoon he suddenly deteriorated neurologically, and a CT scan of the head revealed a massive subarachnoid bleed. The autopsy showed, as cause of death, a subarachnoid hemorrhage due to angioinvasive aspergillosis. In this patient, two foci of aspergillosis were found in the lungs, with focal dissemination to the kidneys and brain. The intrahepatic bile ducts were slightly dilated, and numerous bile infarcts were detected. Although the patient was in renal failure, the kidneys were intact and there was no evidence of immune complex deposition.

The second patient died on the 26th post-transplant day from complications from peritonitis. It is likely that the high doses of corticosteroids used to control early immunologic damage were responsible for poor tissue healing, leading to a leak from the enteric anastomosis.

There were some differences in the immunologic circumstances between the two cases. As mentioned before, the first patient had a splenectomy in 1989 following a motorcycle accident, whereas the second patient still had his spleen, which was subsequently removed 4 days after the xenotransplant.

The first patient was also HIV positive. Our center traditionally does not refuse transplantation to HIV-positive patients [59], but analysis of the immunological parameters obviously differs in the case of an allotransplant. Although this first xenotransplant patient was considered immunocompetent at the time

of the transplant and had no change in this state during his postoperative course [22], it is difficult to judge whether his condition provided a natural immunosuppression, and whether or not this represented an advantage.

After completing the vascular anastomoses the second patient was given an infusion of bone marrow cells from the donor baboon (3×10^8/kg body weight). This was aimed at increasing the natural tolerogenicity induced by the liver transplant [60]. It is believed the liver has certain advantages in immunological terms on account of the large number of dendritic cells it possesses. These cells abandon the transplanted organ and participate in a two-way cell traffic, which gives rise to microchimerism [61, 62]. The autopsy on the first patient confirmed this expectation, since baboon DNA was found (using PCR amplification of baboon-specific DNA) in the patient's heart, kidneys, lungs, and lymph nodes. All blood samples taken from the second patient during the postoperative course showed the presence of xenogeneic baboon DNA.

Analysis of the Two Cases and Strategies For Future Clinical Xenotransplantation

The most disquieting fact in our baboon-to-human liver transplant experience was the disparity between the paucity of the histopathologic findings of rejection (which was very encouraging), and the discouraging functional deficiencies of these xenotransplants, which suggested incomplete control of xenograft rejection. The pathology of the transplanted baboon livers was compared to that of six baboon kidney xenografts transplanted in Denver in 1963 [15, 16]. These kidneys, as we mentioned before, functioned for 10–60 days. The key pathological finding was an occlusive endothelialitis of the graft vessels, presumably antibody mediated. The pathology of those kidneys removed in 1964 [16] showed distal ischemia, caused by the vascular injury, that appeared to be responsible for the patchy gangrene of the xenografts. In the two recent liver xenotransplants, polymorphonuclear leukocytes were seen in the sinusoids immediately after reperfusion, and biliary sludging was detected at the autopsy. Both the sludging and the appearance of polymorphonuclear leukocytes were compatible with a diagnosis of an aborted hyperacute (humoral) rejection. Complement studies were also consistent with this possibility, showing that total complement was depleted for most of the critical first 2 weeks. While the complement system was restored, irreversible damage to the graft may have been already done [9, 22, 23, 53]. Although these baboon liver xenografts looked macroscopically normal, closer inspection showed a very fine microsteatosis, which was particularly obvious in the second patient. This may represent a sub-lethal immunological injury that precluded long-term success in both cases.

We suspect that these livers were acutely damaged by an incomplete version of a form of rejection that was described in 1964 in ABO-incompatible kidneys [63, 64], and seen later in kidney allografts transplanted across a positive lymphocytotoxic crossmatch [17]. These were the first descriptions of hyperacute kidney rejection by preformed antigraft antibodies. A few years later, hyperacute rejection was defined in a more sophisticated way as a complement activation syn-

drome, analogous to the Shwartzman and local Arthus reactions [65, 66]. It was pointed out that, although hyperacute allograft rejection was usually associated with antigraft antibodies, this was not an absolute requirement – a heretical statement at that time. However, the distinction between hyperacute rejection *with* and *without* preformed antibodies is merely the difference between the classical pathway of complement activation, in which the first steps are antibody dependent, and the alternative pathway, which does not require an antibody trigger or the participation of complement components C1, C2, and C4. These hyperacute rejection syndromes, *with* or *without* preformed antigraft antibodies, are not fundamentally different from those seen after xenotransplantation of organs between genetically diverse species [9].

Many methods of manipulating the xenograft recipient have been tried and retried since the 1960s, without any definite success. These methods include antibody suppression [67], antibody depletion [68–79], inhibition of the complement cascade [80–85], and inhibition of the inflammatory response [86, 87]. Alteration of the xenograft before its implantation, mainly by blocking antibody binding sites with recipient $F(ab')_2$ immunoglobulin fragments [76, 88, 89], has also been unsuccessful.

The introduction of the concept of systemic chimerism has heightened interest in designing strategies aimed to alter the cell composition of the graft. The creation of a transgenic pig, to be utilized as a source of organs for clinical xenotransplants, has already been started in a few laboratories [90, 91]. The scientists working on this project have embarked on a program to produce pigs transgenic for human regulators of complement activation (e.g., decay-accelerating factor, DAF; CD59; membrane cofactor protein, MCP). This is achieved by microinjection of human genomic DNA fragments into the pronuclei of fertilized porcine oocytes [92–94]. However, only one of the components of the xenotransplant barrier could be overcome by this strategy (namely, the complement cascade); therefore it is difficult to hope that a complete control of rejection will be achieved by this method alone.

One other extremely fascinating possibility is the production of chimeric organs. Human-to-baboon bone marrow transplantation has already been performed at the Pittsburgh Transplantation Institute laboratories, after conditioning with nonlethal irradiation [95]. In this experiment, two baboons preconditioned with 7.4 Gy total lymphoid irradiation were given unaltered human bone marrow cells at the dose of 6×10^8 cells/kg body weight, without any subsequent treatment. Donor DNA was found widely distributed in the tissues of both animals when killed 18 months later. It is also interesting to note that graft-versus-host disease (GvHD) did not occur in either animal. As recently stated elsewhere [9], "it remains to be seen if incomplete or even full chimerism will change the image of baboon organs enough to make them viewed as allografts by humans."

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