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Liver Transplantation

Procedures and Management

PREFACE

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Hepatic Xenotransplantation: Clinical Experience

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HISTORICAL BACKGROUND AND RATIONALE

The concept of a xenotransplant (transplantation of cells, tissue and organs between different species to create a chimera) is so old that it can be found in Greek and Roman classical mythology. Homer, in describing the centaur Chiron [1], master of Aesculapius, and the Chimera [2] was actually proposing two mythological examples of discordant xenogenic creatures [3]. However, it was only during the early part of this century that a scientific approach was adopted, both experimentally and clinically [4-9], to the question of xenotransplantation. The first attempts were totally unsuccessful, basically because knowledge of pathophysiology, immunology, and even vascular surgical procedures was very primitive 60 to 90 years ago, when compared to what we know now. Consequently, the initial reports of successful (or at least partially successful) clinical xenotransplants date back to the 1960's [10-13]. The organ most frequently involved during these early clinical trials was the kidney. Dialysis was not yet an available therapy, and patients with terminal kidney failure were destined to die from uraemia. In contrast to the liver, kidney transplantation was already well defined from a technical point of view, and a number of successes had been achieved in clinical allotransplantation using immunosuppression based on antimetabolites and steroids [14-19]. The crisis brought about by the lack of donor organs led to these early clinical attempts, as xenotransplantation seemed to be the only operation which could be offered on a large scale to patients with terminally diseased organs. The results achieved by Keith Reemtsma [10] and Thomas Starzl [12] were very encouraging, with patients surviving without dialysis for several months (2 months in the experience of Starzl and up to 9 months for Reemtsma). In 1965, how-ever, dialysis became possible in both Reemtsma's and Starzl's hospitals, and this was accompanied by a simultaneous increase in the number of donor organs available. These two factors lead to the suspension of clinical kidney xenotransplant programs.

Clinical xenotransplants of the heart and liver were occasionally attempted, without success, over the next 20 years [20]. Meanwhile, the refinements of surgical techniques and, above all, the introduction of Cyclosporine into clinical use by Sir Roy Calne [19, 21] created a second organ shortage crisis in the 1980's. In 1984 Leonard Bailey, of Loma Linda, raised the hopes of the scientific community when he transplanted a baboon's heart into a newborn baby weighing 2.6 kg, known as Baby Fae [22]. However, the immunosuppressants used (steroids, Cyclosporine, azathioprine and antithymocyte globulin) were not sufficient to control humoral rejection.

Humoral rejection had actually already been recognized as far back as 1965 as the principal immunological barrier to xenotransplantation [3, 12, 13, 20, 23, 24]. Different procedures for preventing and controlling humoral rejection, both in allo- and xenotransplantation, have been studied since then, many of which have already been discussed by ourselves and others [25-30]. Among the various methods used in the past, two drugs in particular have recently attracted our attention: prostaglandin E_1 and cyclophosphamide.

Treatment with prostaglandins has been shown to be useful in mitigating pathophysiological events linked to humoral rejection in various experimental xenotransplant protocols [27, 31-34]. Furthermore, Quagliata et al. had already demonstrated in 1972 that prostaglandin E has a direct action on the activity of B lymphocytes [34]. Although the liver is traditionally considered to be resistant to humoral rejection [35], there are several examples of antibody induced rejection of liver allotransplants in the clinical

literature [36]. For this reason, we added prostaglandin E_1 to our immunosuppressant protocol for liver allotransplants in 1992, and this allowed us to achieve the same survival results in positive cross-match patients as in negative cross-match ones [37].

Cyclophosphamide is an alkylating agent that blocks the cell cycle in the G_2 phase, and has been widely used in cancer chemotherapy [38]. It is also a powerful immunosuppressant [19] that acts on the humoral [39] and cellular [40-42] limbs of the immune response, being capable of reversing rejection, both experimentally [43-50] and clinically [46, 51-55]. Early successful experience in clinical bone marrow transplantation [56], as well as in a limited number of kidney transplants [57, 58], encouraged its use in a larger series of solid organ transplants, in combination with prednisone and anti-lymphocyte immunoglobulins [51, 55] or, in some cases, azathioprine [51-54].

More recently, Murase et al. achieved 100% long-term survival in a hamster to rat heart xenotransplant model, using a combination of FK506 and cyclophosphamide [59]. The success obtained in this model, combined with the clinical experience accrued during the Sixties, provided the rationale for a new clinical xenotransplant trial at the Pittsburgh Transplantation Institute in 1992. Although the chimpanzee is most likely the best donor in biological terms, due to the very small genetic differences between them and humans, 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 AIDS research [60], and it is estimated that only 70 chimpanzees would be available worldwide as organ donors each year [61]. Therefore, it was decided that the donor would be the baboon (*Papio cynocephalus*), and the organ chosen for this initial clinical trial was the liver, on account of its resistance to humoral rejection [35-37, 62-65]. The pharmacological cocktail used for preventing and controlling rejection was a combination of "old" (steroids, cyclophosphamide and prostaglandin E_1) [19] and "new" (FK506) [66] immunosuppressant drugs.

DONOR SELECTION

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 [12]. All the baboons used during donor selection were *Papio cynocephalus*, and were born in the US [67].

Baboons have group A, B and AB antigens weakly expressed in all cells, with group 0 baboons being extremely rare [68]. However, AB0 incompatibility did not affect the results of previous clinical xenotransplant trials [12, 18]. An AB0 match is desirable in a baboon-to-human xenotransplant, but its absence does not constitute an absolute contraindication. Table 22.1 shows the blood groups of both donors and recipients. Both patients, blood group A and B, respectively, received their liver from compatible donors. In addition to blood group, donor selection criteria included a lymphocytotoxic cross-match, as well as a complete biochemical, viral and bacteriological evaluation of the animals [69]. 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 (STLV, HTLV, SIV, SRV-1, SRV-2, SRV-5, HV-1, HIV-2 and Foamy virus), Herpes viruses (SA-8, HSV, B-virus, rCMV, hCMV, EBV and VZV) and hepatitis viruses (HBV, HAV, and HCV). In addition to this, the animals were examined to exclude tuberculosis and toxoplasmosis, and routine cultures of blood and faeces were performed.

DONOR OPERATION

The donor operation was performed using the traditional technique described by our group [70]. The operations on the donor and the recipient were performed simultaneously, in two different operating theaters. Cold ischemia times were 80 minutes in the first case and 231 minutes in the second. University of Wisconsin solution was used to preserve the organs, as is done in our routine clinical practice.

RECIPIENT OPERATION

The liver xenotransplant was performed using a modification of our standard method, described 30 years ago [71], and employing veno-venous by-pass [72]. The difference in diameter between the vessels of both donor and recipient, and the small size of the

Tab. 22.1. *Blood groups and demographical data of the first 2 cases of baboon-to-human liver transplantation.*

	Blood group (¹)	Age/sex	Diagnosis	Previous surgery	Xenotransplant date	Survival (days)
1	A	35/male	Hepatitis B	Splenectomy	6/28/92	70
2	B	62/male	Hepatitis B		1/10/93	26

(¹) Donor and recipient were of the same blood group.

Fig. 22.1. *Case 2.*
Cholangiogram performed on post-operative day 18, by injection through the percutaneous biliary catheter. This catheter was placed during the xenotransplant operation across the choledochojejunostomy (arrow), and allowed study of the biliary anatomy, as well as daily collection of bile samples after the xenotransplant.

donor's liver (600 cm³ and 450 cm³ in the first and the second cases, respectively) made it necessary to use the piggy-back method [73]. In both cases the right suprahepatic vein of the recipient was closed with a running suture, whilst the left and middle suprahepatic veins were used to perform the suprahepatic vena cava 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 aorta, using a carotid artery interposition graft, in the second.

In the first case, given the considerable 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, the smaller discrepancy made it possible to perform a normal end-to-end portal anastomosis. 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 (3.5 F) diameter catheter was placed into the biliary anastomosis and brought out through the abdominal wall (Fig. 22.1), in order to gain direct access to the bile duct, to study its anatomy and take bile samples during the post-operative period.

IMMUNOSUPPRESSIVE THERAPY

The immunosuppressive protocol comprised 4 drugs: cyclophosphamide, FK506, methylprednisolone and prostaglandin E_1. The cyclophosphamide was started 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/day. FK506, steroids, and prostaglandin were started on the day of the transplant, using the same protocol we follow in clinical liver allotransplantation. A detailed description of the immunosuppressive drug dosing, and blood levels obtained, was recently published [74, 75].

CLINICAL COURSE

As of this writing, there have been 2 clinical baboon liver xenotransplants, performed on June 28, 1992, and January 10, 1993, respectively. The first patient was extubated 17 hours after the operation, and he lived for 70 days mostly in a regular hospital ward, with a relatively normal quality of life. The second patient, who was much older (see table 22.1), never regained a level of consciousness sufficient to allow him to be weaned off the mechanical ventilator, and survived for only 26 days. Figures 22.2 and 22.3 describe the post-operative courses, as pertains to the transplanted liver function.

The first patient underwent 5 liver biopsies, while the second had 7. None of the biopsies showed acute cellular rejection, according to the criteria routinely used in liver allotransplantation [74-76]. However, direct immunofluorescence demonstrated the presence of endothelial deposits of immunoglobulins (IgG>IgA>IgM), and complement (particularly Clq) in both cases.

Macroscopically, considerable hepatic regeneration was noted in both cases, with a significant increase in the volume of the baboon organs. Figure 22.4 shows the organ transplanted into the first patient, during the course of an open liver biopsy performed on July 10, 1992, 12 days after the xenotransplant. The normal multilobar appearance of the baboon liver is evident, as is the increase in volume when compared with its original size (600 cm³). Computerized tomography was used to calculate the volume of the transplanted livers [77]. Both livers showed an extremely rapid volumetric growth, as normally occurs when a human liver is transplanted into a recipient with a larger abdomen than the donor [78]. Figures 22.5 and 22.6 show the computerized tomographies performed on the two recipients, on the 26th and the 14th post-operative days, respectively. The first patient's liver grew from an initial volume of 600 cm³ to 1,555 cm³ in 26 days. The second patient's liver grew from an initial volume of 450 cm³ to 1,741 cm³ in 14 days.

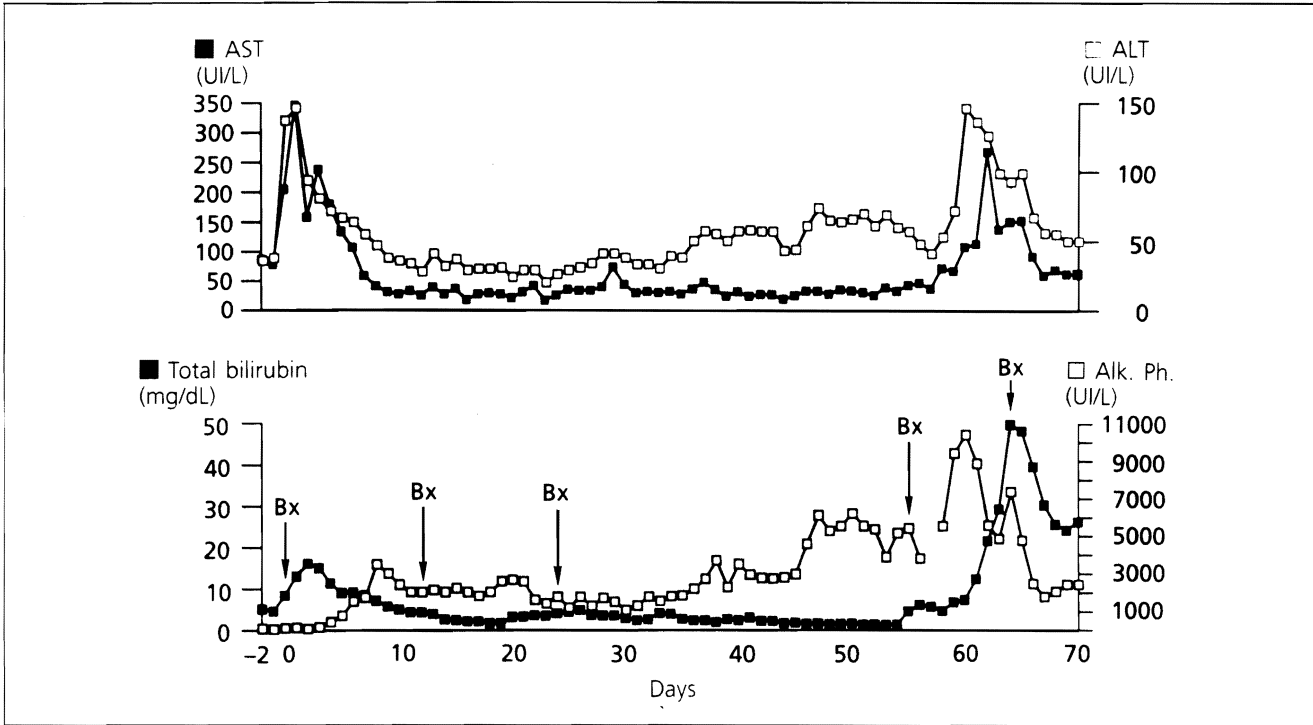As mentioned above, cellular rejection was not a

Fig. 22.2. *Case 1.*
The graph shows alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), alkaline phosphatase (Alk. Ph.), and to-
tal bilirubin values during the post-operative course. Bx shows the number of liver biopsies, and the days when they were
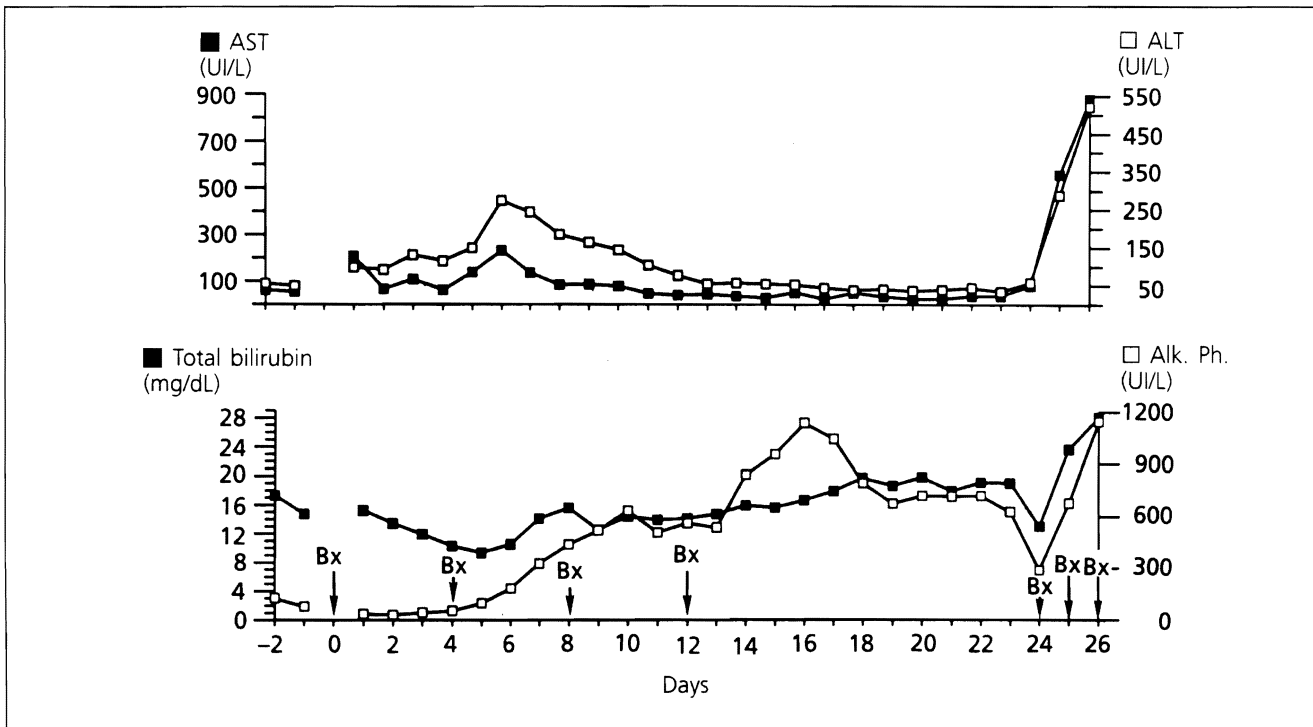performed.

Fig. 22.3. *Case 2.*
The graph shows alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), alkaline phosphatase (Alk. Ph.), and to-
tal bilirubin values during the post-operative course. Bx shows the number of liver biopsies, and the days when they were
performed.

Fig. 22.4. *Case 1.*
Intraoperative photograph taken 12 days after the xenotransplant, when the patient was explored to perform an open liver biopsy. The normal multilobar anatomy of the baboon liver is clearly appreciated.
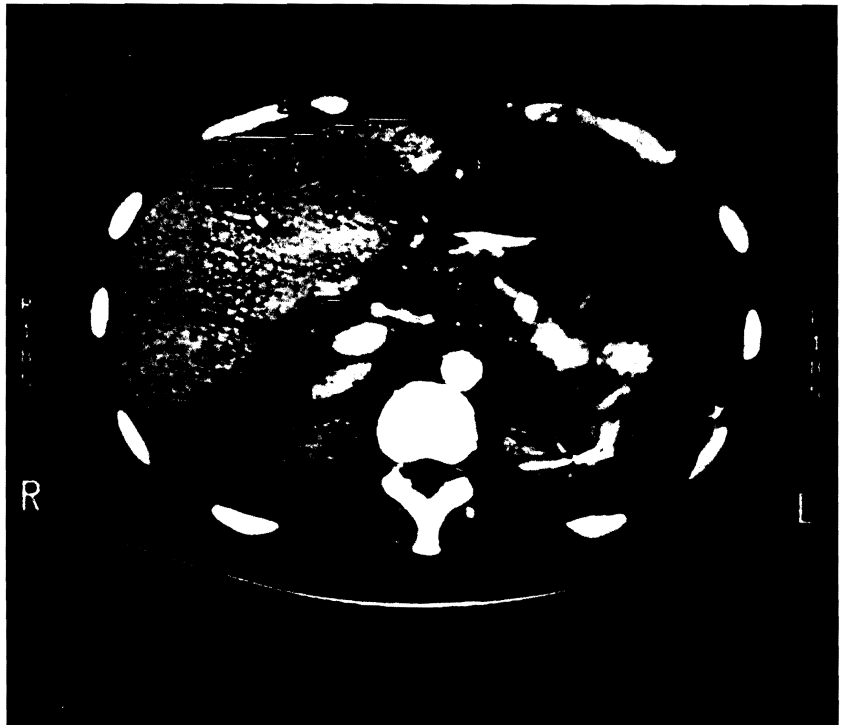

Fig. 22.5. *Case 1.*
Abdominal CT on post-operative day 26. The liver volume has increased to 1,555 cm³, from an initial value of 600 cm³.

problem with either patient. The pharmacological cocktail used (cyclophosphamide, FK506, methyl-prednisolone and prostaglandin E₁) prevented the immunological lesions characteristic of xenotransplantation [20].

The effectiveness of these drugs in the experi-

mental concordant xenotransplant [59, 79] was also confirmed in our clinical experience. The cause of death in the first patient was a subarachnoid hemorrhage, caused by angioinvasive aspergillosis.

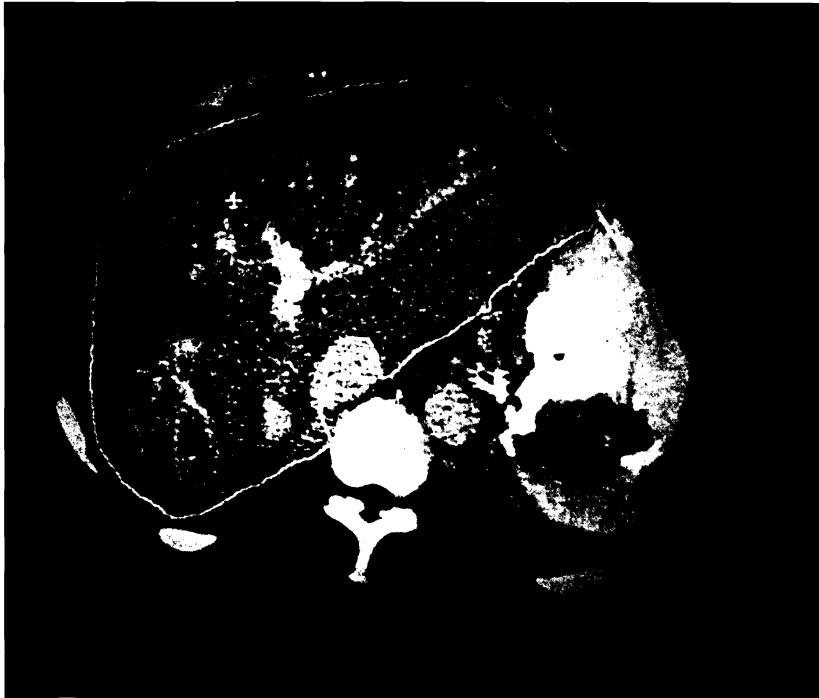The second patient's death was due to bacterial sepsis.

Fig. 22.6. *Case 2.*
Abdominal CT on post-operative day 14.
The liver volume has increased to 1,741
cm³, from an initial value of 450 cm³.

ANALYSIS OF RESULTS

THE CHOLESTASIS ENIGMA

From an immunopathological point of view, we paid particular attention to the presence of T lymphocytes (CD4+ < CD8+) and NK cells in the basal membrane of the biliary canalicula. This is due to the fact that both patients showed definite signs of intrahepatic cholestasis with a virtually intact hepatocellular architecture. As can be seen in Figure 22.2, the first patient had a normal serum bilirubin for much of the post-operative course, whereas the alkaline phosphatase was always very high. Alkaline phosphatase values in the second patient were not as conspicuously high as in the first (Fig. 22.3), although they were always above normal limits. Serum bilirubin in the second patient never reached a normal value, remaining above 8 mg/dL. Whereas the post-mortem examination revealed the presence of a large amount of biliary sludge in the first patient, possibly linked to an obstructive problem, the transanastomotic biliary catheter in the second patient excluded mechanical factors as the aetiology of the increase in canalicular enzymes.

which was subsequently removed 4 days after the xenotransplant. The first patient was also HIV-positive. Although he was considered immunocompetent at the time of the transplant, and had no changes in this state during his post-operative course [74], it is difficult to judge whether his condition provided a natural immunosuppression, and whether or not this represented an advantage. Our centre traditionally does not refuse transplantation to HIV-positive subjects [80], but analysis of the immunological parameters obviously differs in the case of an allotransplant. After completing the vascular anastomoses the second patient was given an infusion of bone marrow cells from the donor baboon. This was aimed at increasing the natural tolerogenicity induced by the liver transplant [81]. 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 [82, 83] (see also Chapter 25). The autopsy on the first patient confirmed these expectations, since baboon DNA was found in the patient's heart, kidneys, lungs and lymph nodes. All blood samples taken from the second patient during the post-operative course showed the presence of xenogeneic DNA.

BIOLOGICAL DIFFERENCE

The two cases were basically different from an immunological viewpoint. The first patient had a splenectomy in 1989 following a motorcycle accident, whereas the second patient still had his spleen,

METABOLISM

In addition to immunological problems, clinical liver xenotransplant also has important metabolic aspects. A baboon liver transplanted into a human continues to produce the donor's phenotypical pro-

teins. This concept is the basis on which many congenital metabolic abnormalities are solved by allotransplantation of the liver [84, 85]. It follows that liver xenotransplant creates a baboon-specific hepatic metabolism in the recipient. This aspect was already clear to us from previous studies performed on the hamster-to-rat liver xenotransplant model. Although both are rodents, the phylogenetic distance between a hamster and a rat is put at somewhere between 15 and 40 million years [86]. Analysis of the coagulation proteins showed large differences between the two species of rodents and, when the hamster liver was transplanted to the rat, the latter's coagulation profile changed radically, becoming comparable to that of the donor animal [87]. Despite this, the recipient did not suffer from a hemorrhagic diathesis. Similar variations occurred in the baboon-to-human liver xenotransplant [74, 88], where the recipient acquired the same coagulation profile as the baboon, while retaining a normal prothrombin time and coagulation capacity [74, 88].

A transplanted baboon liver also continues to produce species-specific complement. This could provide immunological protection of the xenotransplant, since the complement produced by the baboon liver should not be involved in rejection of the liver which produces it. The baboon liver could theoretically introduce lethal alterations into the human metabolic pathways, but this was not the case in this experience. We did see numerous changes involving, for example, the metabolism of purines, albumin, cholesterol and triglycerides, without this causing any deleterious effects to the host organism. However, all these aspects obviously require further detailed investigation. The metabolic issues surrounding clinical xenotransplantation could well turn out to be a Pandora's box.

CONCLUSIONS

Our experience to date is still too small to allow conclusive scientific assertions. Only through further work will we be able to determine the advantages and feasibility of this fascinating therapeutic option. However, the hope of having access to an unlimited number of donors is a strong incentive for proceeding in this direction. The Pittsburgh Transplantation Institute believes that current knowledge of rejection immunopathology, and the immunosuppressant drugs at our disposal, justify clinical trials of xenotransplantation. Starting from this premise we notified, in November, 1991 the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institute of Health (Jay Hoofnagle, MD, and Philip Gordon, MD), the Food and Drug Administration (Ron Lieberman, MD, and Gregory Burke, MD) and the Secretary of Health and Human Services (Louis Sullivan, MD) that we intended to proceed with the clinical liver xenotransplant project. Eight months were then needed to present the scientific documentation in our possession to the appropriate government agencies in the US, our Institutional Review Board, the Ethics Committee of the University of Pittsburgh Medical Center, and the members of the US Congress. In March, 1992, we also brought together a committee formed by six eminent European and American surgeons, coordinated by Keith Reemtsma of Columbia University, to hear the opinions of other experts before proceeding with the first baboon liver xenotransplant.

After making several modifications to our initial protocol, on the basis of suggestions made by the various experts consulted, we performed the first xenotransplant on June 28, 1992, and the second on January 10, 1993. During the long interval between the first and the second, despite the fact that we had the authorization to perform 4 consecutive liver xenotransplants [89], we chose to bring the same group of experts previously consulted together again, this time at the New York Academy of Medicine, so that they could analyze the results obtained in the first xenotransplant. On this occasion, we were advised to continue the clinical trial.

A program of this kind obviously raises ethical problems, in addition to those of a strictly medical nature. Certain ethical movements consider this project to be immoral [90]). We do not consider this the appropriate place to enter into the dispute between the supporters of interspecies equality (a modern equivalent of Jainism [90], supporters of species inequality, and "speciesists" [92]. However, we believe we can share the sentiments of Stephen Post [90], who stated that the Pittsburgh project «has successfully reminded us that the human good remains appropriately the highest good, despite the cultural inroads of anthropomorphism».

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