SURGICAL TECHNOLOGY INTERNATIONAL II

INTERNATIONAL DEVELOPMENTS IN SURGERY AND SURGICAL RESEARCH

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#1672

Liver Xenotransplantation

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DURING THE PAST 30 YEARS ORTHOTOPIC LIVER TRANSPLANTATION HAS BECOME A highly successful form of surgical treatment.[1-3] The significant advances achieved in this field have led to an increased demand for organs and created a wide gap between organ availability and organ supply.[4]

A wider availability of organs for transplantation would allow an expansion[5] rather than a contraction of the indications for transplantation, and, at the same time a relaxation of the patient selection criteria.[6,7] All these facts clearly justify the renewed interest observed in the last decade in xenotransplantation.[8]

The original concept of xenografting, meaning the transplantation of cells, tissues, or organs between different species, is so ancient that it is easily recognizable in Greek and Roman mythology. The centaur Chiron,[9] the teacher of Esculapius, and the Chimera[10] are legendary examples of discordant[11] xenogeneic creatures.

However, it is only during this century that scientists have been able to bring this idea into the clinical arena. The early efforts were prompted by the shortage of humans organs at a time when there were few alternatives for treating end-stage organ failure.[12-24]

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.[12,13] 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[14] a lamb was used as a kidney donor and the patient died after 9 days. On February 16, 1963, Hitchcock of the Hennepin County Hospital in Minneapolis, transplanted the kidney of a baboon to a 65-year-old woman. The organ functioned for 4 days before its artery clotted.[15] A few months later on October 8, 1963, Reemtsma of Tulane University used a Rhesus monkey as a kidney donor for a human recipient who survived 12 days. Then, Reemtsma tried again with a series of 6 consecutive chimpanzee kidney grafts.[16] One of these xenografts functioned for 270 days.

In December 1963 and January 1964, six patients were transplanted using baboon kidneys at the University of Colorado in Denver.[18] All of these kidneys worked immediately and sustained a dialysis-free life for 10 to 60 days. The patients were on heavy doses of azathioprine and prednisone and in 4 of them sepsis was the leading cause of death, while rejection was mainly responsible for the other two deaths. However, the rejection pathology was not qualitively different from that observed in allografts.[25] In 1984, similar immunopathological events caused fail-

Aided by Research Grants from the Veterans Administration and Project Grant No. DK 29961 from the

ure of a baboon heart transplanted in a 2,200 gr neonate, known as Baby Fae,[24] after 20 days. Despite heavy cyclosporine-steroid immunosuppression.

A pig kidney and a pig heart transplanted respectively by Kuss[26] and Ross[21] in the '60s were hyperacutely rejected in a matter of minutes, clearly demonstrating that the pig was not and will not be an easy donor for a human recipient.

The Antiproliferative Drugs

Sir Peter Medawar, in 1969, stated that: "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".[27] However, the laboratory work performed at different institutions in the following 15-20 years did not bring particularly encouraging results in further clinical trials. In May 1992 the results of a study performed in Pittsburgh by Dr. Noriko Murase et al.[28] were discussed at the meeting of the American Society of Transplant Surgeons in Chicago. Murase's work clearly showed, in a hamster-to-rat xenotransplant model, that when FK506 treatment was combined with either of two "antiproliferative" drugs that suppress purine (RS 61443) or pyrimidine (Brequinar) ribonucleotide synthesis for the first two post-transplant weeks, indefinite survival under continued FK506 alone was routinely achievable. The use of cyclophosphamide, an alkylating agent with considerable B cell specificity,[29,30] 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 daily administration of FK506 only. When cyclophosphamide had been used in the past for kidney and intestinal transplants in the dog, there was no prolongation of graft survival, or else the effect was a minor one.[31-33] The dog may have been an inappropriate model to evaluate cyclophosphamide for human immunosuppression.[31-33] How-ever, the successful use of this drug by Santos et al.[34,35] in bone marrow grafts, and by other authors in a few cases of clinical kidney transplantation,[36,37] prompted its use at the University of Colorado in a series of liver and kidney transplant patients, as the baseline for chronic therapy, in combination with prednisone and horse antilymphocyte globulin.[38,39] Later, a more extensive report was published again by the Denver group.[40] This previous clinical use of cyclophosphamide as an effective drug in transplant patients appeared to justify its use in clinical xenograft trials.[28]

Clinical Trial

On June 28, 1992, and on January 10, 1993, two patients aged 35 and 62 years respectively, suffering from end stage liver disease related to hepatitis B virus (HBV) underwent a liver xenotransplantation (Table 1). The chosen donor was the baboon **Papio cynocephalus**. Theoretically, at least 3 animal-human combinations

BLOOD GROUP AND DEMOGRAPHICAL DATA OF THE 2 FIRST CASES OF BABOON-TO-HUMAN LIVER XENOGRAFT

	ABO (BABOON)	ABO (PATIENT)	PATIENT DATA	DIAGNOSIS	PREVIOUS ABDOMINAL SURGERY	XENOGRAFT DATE	SURVIVAL (DAYS)
1	A	A	35y male	Hepatitis B	Splenectomy	6/28/92	70
2	B	B	62y male	Hepatitis B	None	1/10/93	26

Table 1.

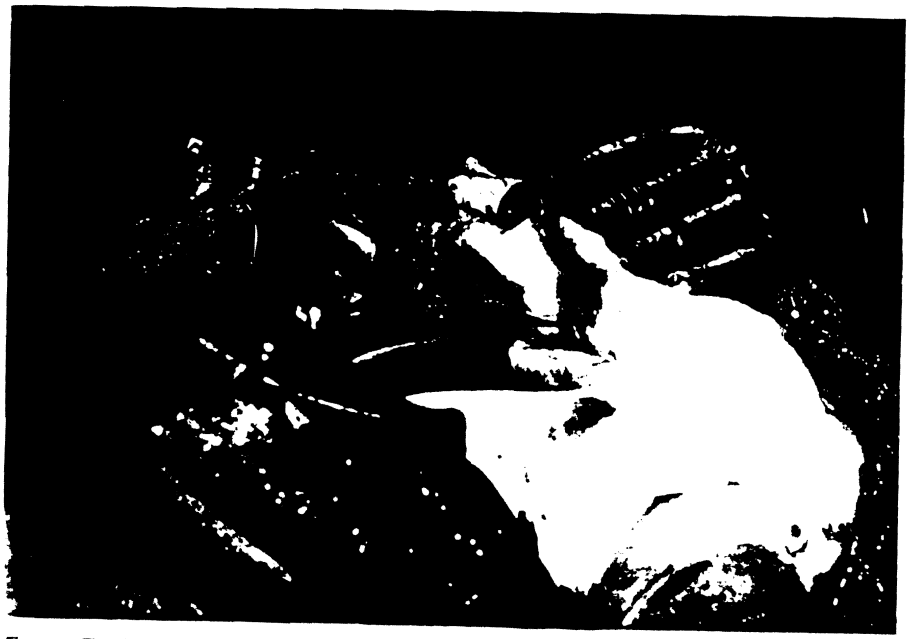

Figure 1. The Papio cynocephalus liver at the time of reperfusion in the baboon-to-human liver xenotransplant no. 1. The organ is uniformly and nicely reperfused.

Figure 2. Baboon-to-human liver xenotransplantation. Case 2. Cholangiogram performed on post-operative day 18, through the indwelling biliary catheter placed and exteriorized at the time of the surgery. The arrow shows the choledochojejunostomy on a roux-en-Y bowel loop. The biliary catheter placed in Case 2 allowed routine collection of the bile during the post-operative course. (From: Marino IR, Tzakis AG, Fung JJ, Todo S, Doyle HR, Starzl TE: Xenotrapianto epatico: esperienza clinica. In: Il Trapianto di Fegato (DF D'Amico, N Bassi, eds) pp. 269280, Masson, Milan, Italy, 1993. Used by permission).

possibly qualified for this xenotransplantation attempt. The Rhesus monkey-to-human, the baboon-to-human, and the chimpanzee-to-human. The Rhesus monkey had been used in 1963 by Reemtsma for an heterotopic lollipop kidney transplant. The Rhesus kidneys were aggressively rejected in a few days.[16] The use of the baboon at the University of Colorado allowed a dialysis free survival of the uremic patient for up to 60 days, and only two of the 6 patients transplanted died from rejection. The chimpanzee was used at Tulane University again by Dr. Reemtsma and one of his patients lived for nine months without dialysis, and succumbing only to an acute illness of undetermined etiology (probably pneumonia). Consequently, on the basis of the previous experience the chimpanzee could seem the best possible donor. However, it is an endangered species and in the U.S. only between 25 and 50 chimpanzees per year would be available for all biomedical research, including that in the important fields of hepatitis and AIDS.[41] Also, it has been calculated that only 70 chimpanzees could be available world wide as organ donors.[42] The use of chimpanzees would further jeopardize this species without solving the organ shortage problem.

The baboon, thus, appeared to be the only possible donor choice, and the liver seemed to be the organ to use in the clinical trial, because its relative resistance to humoral rejection.[43-49]

The pharmacological cocktail used for the prevention and the control of rejection was made by "old" immunosuppressants (steroids, cyclophosphamide and Prostoglandin E_1),[1,2,29-35,38-40,44,50] and "new" immunosuppressants (FK506).[51]

Donor Surveillance and Selection

The baboons for the donor selection were provided by the Southwest Foundation for Research and Education (SFRE), San Antonio, Texas, who also provided the baboons for the previous Denver series of baboon-to-human renal xenotransplantation.[18] All of the baboons involved in the donor selection process were Papio cynocephalus, and were born in the U.S.A. at SFRE.[52] Baboons have weakly expressed A, B, and AB blood types on all cells, and will rarely have type O blood group,[53] but ABO incompatibility did not influence the outcome in previous human xenograft trials (1,18). Thus, ABO match in baboon-to-human xenotransplantation is desirable but not mandatory. However, both of our patients had ABO compatible donors: A to A in Case 1, and B to B in Case 2 (Table 1).

The conventional lymphocytotoxic crossmatch of the recipient sera to their donor lymphocytes was positive in both cases but negative after dithiothreitol treatment. During the selection process the baboons underwent a complete biochemical and infectious disease work-up. This work-up was performed at the Virus Reference Laboratory of SFRE and included screening for retroviruses (STLV, HTLV, SIV, SRV1, SRV2, SRV5, HIV1, HIV2, and foamy virus), Herpes viruses (SA8, HSV, BVirus, rCMV, hCMV, EBV, and VZV), and hepatitis viruses (HBV, HAV, and HCV), Marburg virus, encephalomyocarditis virus, lympho-

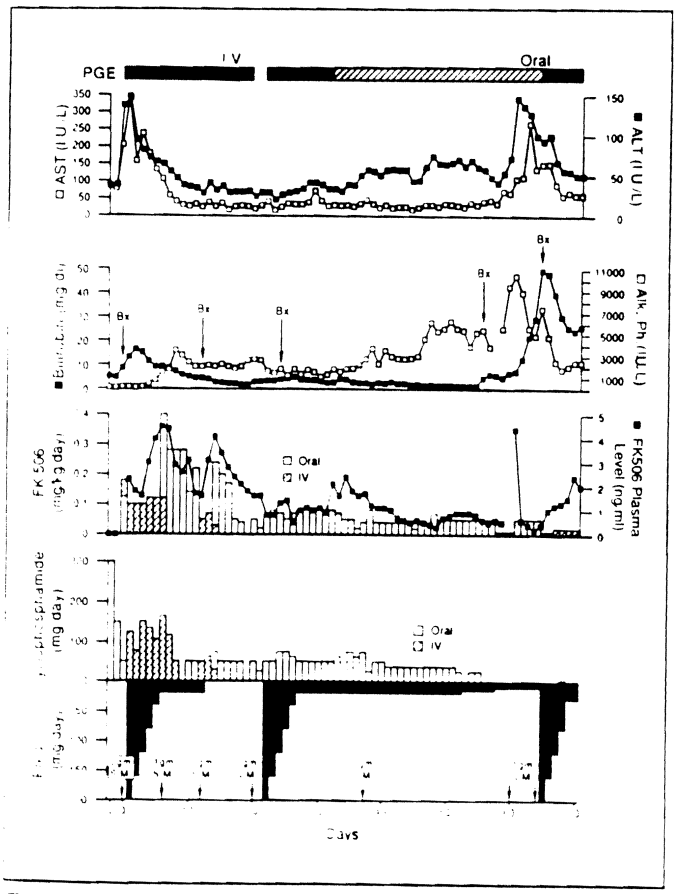

Figure 3. Clinical course after baboon-to-human liver transplant. Case 1. SM, SolumedrolÆ (methylprednisolone); PGE, prostaglandin E_1; Bx, liver biopsy; AST, aspartate aminotransferese; ALT, alanine aminotransferase; Alk Ph, alkaline phosphatase.(from: Starzl TE, Fung J, Tzakis A, Todo S, Demetris AJ, Marino IR, Doyle H, Zeevi A, Warty V, Michaels M, Kusne S, Rudert WA, Trucco M: Baboon to human liver transplantation. Lancet 341(8837):6571, 1993. Used by permission.)

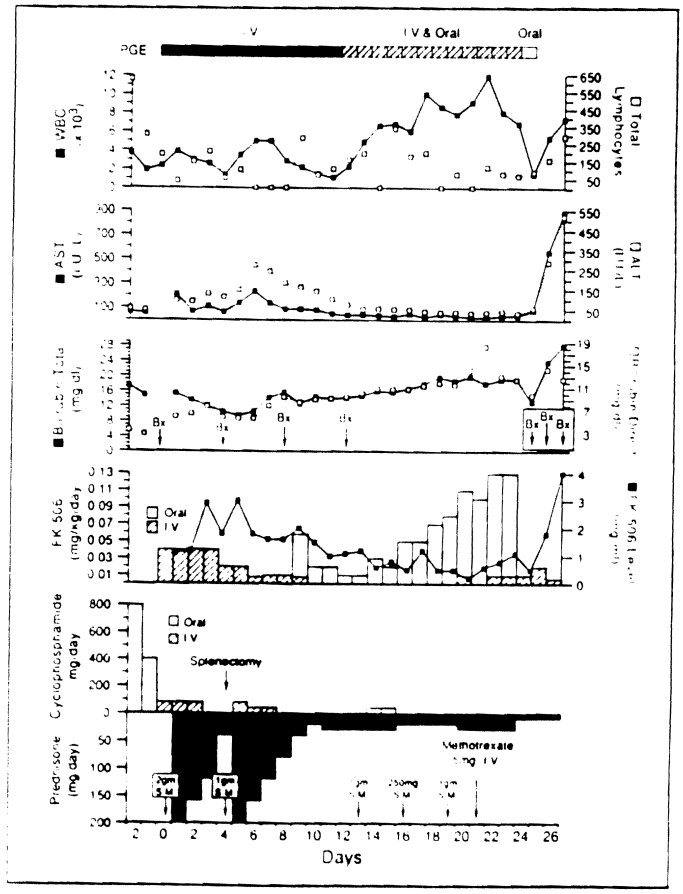

Figure 4. Clinical course after baboon-to-human liver transplant Case 2. SM, SolumedrolÆ (methylprednisolone); PGE, prostaglandin E_1; Bx, liver biopsy; AST, aspartate aminotransferese; ALT, alanine aminotransferase; WBC, white blood cell count. (from: Starzl TE, Tzakis A, Fung JJ, Todo S, Marino IR, Demetris AJ: Human liver xenotransplantation. Xeno, in press. Used by permission.)

choriomeningitis virus, and hemorrhagic fever virus. The animals were also screened for tuberculosis and toxoplasmosis and blood and stool cultures were obtained.

Both Papio cynocephalus donors were healthy and found to be antibody positive for foamy virus, EBV, CMV, and Simian agent 8. In addition the donor to the first patient had VZV antibodies. The donor/recipient weights were 25.8/70 Kg in Case 1 and 35.8/80.4 Kg in Case 2.[54,55]

Donor and Recipient Operations

The donor operation for the liver harvesting from the two baboons was performed according to our donor standard technique.[56] The donor and recipient operations were performed in two different operating rooms, with the donor operations starting slightly after the recipient operations to minimize the cold ischemia time (137 minutes in Case 1, and 231 minutes in Case 2). The baboon donor livers were preserved with the University of Wisconsin solution.

Both baboon-to-human liver xenotransplantations were performed using a piggyback technique,[57] and a venovenous bypass,[58] a modification of the standard technique for orthotopic liver transplantation described 30 years ago.[59] Specifically due to the small size of the donor livers (600g and 450g, respectively in Case 1 and Case 2), and the size discrepancy between the recipient and the donor vessels, the recipient's right hepatic veins were closed with a running suture, and the donor upper cava was anastomosed end-to-end to an adequate cuff fashioned using the recipients middle and left hepatic veins. The donor celiac axis was anastomosed end-to-end to the recipient common hepatic artery in Case 1, and end-to-side on the supra celiac recipient aorta in Case 2, by interposition of a donor carotid graft. The donor portal vein was anastomosed on the stump of the recipient left portal vein in Case 1, while the size match allowed a direct porta-to-porta anastomosis in Case 2. The liver reperfused promptly and uniformly (Figure 1) in both cases. The post-reperfusion biopsies showed a good liver architecture, with a moderate degree of sinusoidal neutrophilic aggregates. The biliary reconstruction, with a Roux-en-Y choledochojejunostomy in both cases. However, in Case 2 an indwelling biliary catheter was placed at the time of the operation and exteriorized. This catheter (Figure 2) allowed routine collection of the bile during the post-operative course of the second patient.

Postoperative Course

The immunosuppression was based on the use of 4 drugs: cyclophosphamide, FK506, methylprednisolone and PGE_1. Doses and routes of administration are reported in Figure 3 and 4. Cyclophosphamide was started 2 days before the xenotransplants, and was given for 56/70 days in Case 1 and 10/26 days in Case 2, at a dose ranging from 0.07 to 10.6 mg/kg/day. FK506 was started the same day of the xenografts and, except for higher doses given during the first 2 postoperative weeks in Case 1, the doses were within standard therapeutic ranges. Detailed descriptions of the immunosuppressive drug doses and of their blood levels have been recently reported elsewhere.[54,55,60]

Some of the demographic characteristics of the 2 patients are shown in Table 1. Both patients had end-stage chronic active hepatitis caused by hepatitis B virus (HBV). The evidence that the baboon liver would be resistant to the HBV[54] that reinfects most allografts under comparable circumstance[61] promoted the selection of these two candidates who had already been refused human liver transplantation at other institutions. Some differences between the two patients could have impacted on the efficacy of perioperative immune modulation. The second patient was nearly twice as old and far more frail than the first patient. Also, the second patient did not have a splenectomy, and the spleen was removed on day postoperative 4. The first patient underwent splenectomy in 1989 after a motorcycle accident. Both patients were immunocompetent at the time of the xenograft, although the first also had an HIV infection.[54] Both patients were in stage 3 coma during the 24 hours preceding the surgery. The first patient woke promptly, was extubated after 17 hours, and was eating and walking within 5 days. He had an almost normal bilirubin for the majority of the 70 days of survival (Figure 3). He also spent almost 30 days in a regular ward. The second patient, by contrast, remained icteric (Figure 4), and comatose and was mechanically ventilated for the 26 days of survival. Both patients suffered from hypoalbuminemia and received frequent albumin infusion.[54,55] The first patient went into renal failure on postoperative day 21, while the second patient became anuric immediately after surgery.

Papio cynocephalus normally produce elevated levels of factor VII and low levels of factors IX and XI, as compared t humans. Coagulation profiles were done in both recipients preoperatively and several times postoperatively. Our results, reported elsewhere,[62] demonstrated that the baboon's coagulation pattern was acquired by the patient after liver xenografting. This fact, however, did not

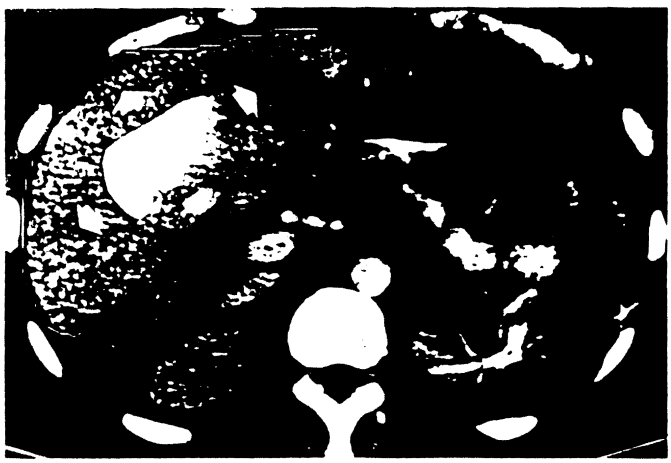

Figure 5. Baboon-to-human liver transplant. Case 1. CT scan of the abdomen performed on postoperative day 24. The CT-calculated liver volume was 1,555cc. The white insert shows the original volume of the baboon liver on the day of the transplant (600cc).

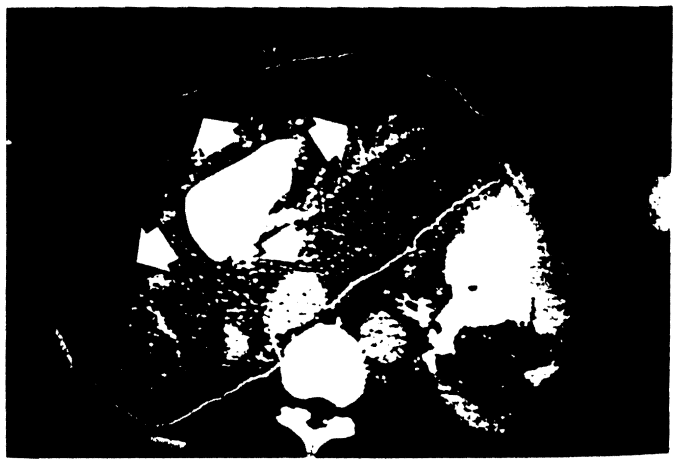

Figure 6. Baboon-to-human liver transplant. Case 2. CT scan of the abdomen performed on postoperative day 14. The CT-calculated liver volume was 1,741cc. The white insert shows the original volume of the baboon liver on the day of the transplant (450cc).

affect the clotting ability of the patients.

During their postoperative courses the patients underwent several liver biopsies (6 in patient 1 and 8 in patient 2, including the autopsy specimens). Only the biopsy obtained from the first patient on the 12th postoperative day had signs of mild focal cellular rejection, while no evidence of cellular rejection was detected in any of the other biopsies from either patient.[54,55,63] No evidence of HBV reinfection was detectable by immunoperoxidase staining in the liver tissue at any time in the two patients.

Both baboon livers underwent a dramatical regeneration after the xenograft, with tripling or quadrupling the organ volume within the first month. Figure 5 and 6 show the change in size of the livers after the xenografts, calculated by CT scan, with the standard method routinely used in our liver patients.[64]

The four drug immunosuppressive cocktail (FK506, methylprednisolone, PGE_1, and cyclophosphamide) prevented these two liver xenografts from being destroyed by cellular as well as humoral rejection. The role played by cyclophosphamide and other "antiproliferative" drugs in the Murase's experiments[28] reported above, was largely confirmed by this clinical trial.

The cause of death was diffuse subarachnoid hemorrhage and left uncal brain stem herniation, secondary to angioinvasive aspergillosis in Case 1, and sepsis from peritonitis in Case 2. Details of the necropsies have been reported elsewhere.[54,55]

Immunologic and Metabolic Questions

The liver, in the hamster-to-rat xenograft model, demonstrated a unique ability to resist rejection more than other organs[28], and was also found to be able to shield other concomitantly transplanted organs from rejection.[65] Also, the liver can resist the attack of preformed xenoantibodies.[65] The elevated amount of dendritic cells and other passile tissue leukocytes able to leave the transplanted organ and induce systemic microchimerism makes the liver immunologically privileged. The production of this cell chimerism is, in fact, the basis of organ transplant acceptance and tolerance.[66] Cell chimerism was proven in the first patient, where baboon DNA was found in many organs at necropsy, including the heart, lung, kidney and lymph nodes. Augmentation of this cell migration was attempted in the second patient by infusion of the donor baboon bone marrow cells after the reperfusion of the liver. All the blood samples collected for this purpose during his postoperative course demonstrated clearly evidence of cell chimerism by PCR.

A baboon liver transplanted in a human being continues to synthesize protein with the donor phenotype. This concept is the basis for liver allotransplantation in many human congenital metabolic diseases.[67,68] Thus, a baboon-to-human liver xenograft creates in the recipient a baboon-specific hepatic metabolism. This fact was already demonstrated in the hamster-to-rat combination, two rodents philogenetically far different for 15-40 million years.[69] The study of clotting factors in these two animals showed significant differences, and when a rat received a hamster liver its coagulation profile became identical to the donor pattern.[70] However, the recipient rat does not suffer from any coagulative diathesis.

Similar changes happened in the human xenografts with changes in the metabolism of hepatic based clotting factors, albumin, several globulins, uric acid, and cholesterol.[54,55] The survival of the two patients was too short to accurately state whether or not these events could be the source of a long term metabolic baboon/human incompatibility.

The other open question is related to the complement role in these clinical xenotransplants. It has been shown that total complement was significantly depleted in the first 10 postoperative days while C3, C4 and C5 were undetectable in the first 14 days.[54,55] Also, during the first postoperative 8 days circulating antigen-antibody complexes were present. After this initial phase the total complement returned to almost a normal concentration. The important role of complement activation with neutrophil participation was emphasized 25 years ago by revealing the analogy of hyperacute kidney rejection with the Shwartzman and Arthur reactions.[71,72]

Experiments aimed to block the pathogenicity derived by the cleavage of C3 and C5 are being currently conducted in the laboratories of the Pittsburgh Transplantation Institute.

Conclusion

It is obvious that a project of this nature raises problems that leave the medical area and directly involve ethical issues. Few ethical movements believe that such a project is unethical.[73] We do not think that this is the right place for opening a debate between advocates of interspecies equality, a modern lainism,[74] believers of interspecies inequality, and speciesists.[75] However, we appreciate and share the feelings of Stephen Post[73] 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." ♦*STI*

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