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Transplantation of the Liver

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Xenotransplantation: Principles and Practice

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The concept of using animal parts to save human life is bathed in ancient mythology. The Hindu religion notes that the elephant-headed god of wisdom, Lord Ganesha, was created by transplanting the head of an elephant on the traumatically beheaded child of Lord Shiva and his wife Parvati. Lord Ganesha is primarily worshipped as the god who removes obstacles and leads to success. Yet the field of xenotransplantation has not been rewarded by long-term clinical success, primarily related to the lack of understanding of the pathophysiology of xenotransplant rejection and, therefore, methods to control rejection.

The principal driving force in the development of the field of xenotransplantation has been the lack of suitable donors for human transplantation. Isolated case reports of using animal kidneys appeared in the early 1900s from sources including pig, goat, nonhuman primate, and lamb.[1] With the discovery of the immune nature of organ rejection by Medawar[2] and Snell[3] in the 1940s, much of the emphasis in organ transplantation focused on means to suppress the immune response. The development of 6-mercaptopurine by Schwartz and Dameshak[4] provided the impetus for transplantation of foreign tissue. The application of azathioprine to allograft transplantation resulted in realization of the success of allotransplantation,[5] although limited by significant rejection episodes with graft and patient loss. It was not until the development of more effective immunosuppression (such as with cyclosporine) that the magnitude of the limitation of azathioprine was apparent. Nevertheless, in the 1960s, a number of subhuman primate-to-human kidney transplants were attempted. Even with this relatively ineffective form of immunosuppression, function of subhuman primate (chimpanzee) xenografts could be demonstrated (in one patient up to 9 months after transplantation).[6] In 1963, seven patients received baboon kidneys, all of which functioned immediately.[7, 8] These heterografts maintained dialysis-free function for up to 60 days. However, in spite of the high doses of azathioprine and prednisone, the grafts were eventually rejected.

In 1968, the guidelines for defining brain death were published in the *Journal of the American Medical Association*.[9] 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 the government financing of the end-stage kidney 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.

Refinements of surgical techniques, improvement in immunosuppression, development of effective organ preservation, and broadening indications for transplantation have pushed the success of transplantation into the realm of acceptability, with transplantation being the treatment of choice for many patients with end-stage organ disease. Yet the success of allograft transplantation has again highlighted the consequences of organ shortages, as witnessed by the increasing number of deaths occurring while waiting for transplantation. This is particularly highlighted in certain candidate populations, such as children, in whom donor scarcity is even more apparent. Such was the rationale for the only attempt at xenotransplantation in the cyclosporine era. The shortage of neonatal hearts for treatment of severe congenital cardiac anomalies prompted Dr. Leonard Bailey to use a baboon heart for transplantation into an infant ("Baby Fae") with a hypoplastic left ventricle in 1983.[10] Although that immunosuppressive regimen included cyclosporine, the heart was eventually rejected by antibody-mediated mechanisms 20 days after transplantation. No further attempts at xenotransplantation were done for almost a decade, until three attempts at liver xenotransplantation were reported in 1992 and 1993.

PATHOLOGY

The pathology of the rejection process was not well understood during the early era of xenotransplantation, although the descriptions of the rejected xenograft kidneys

from both baboons and chimpanzees are consistent with the pathophysiology of xenograft rejection as we understand it today. Lymphocytotoxic antibodies were first recognized in 1965[11] as a cause for antibody-mediated rejection. In the baboon-to-human kidney xenotransplant cases just described, heterospecific antibodies could be detected bound to the kidney xenografts.[7, 12] 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 noted that the rejected xenografts showed variability in the histology, from total infarction to cellular infiltrates to interstitial edema and tubular necrosis to only intimal hypertrophy. The antibody component of rejection has been the central issue of xenotransplantation since that time.

Calne developed the terms *discordant* and *concordant xenotransplantation* to distinguish between different types of rejection that occurred after cross-species transplantation.[13] Calne suggested that cellular rejection would be the the primary cause of graft loss in concordant xenotransplants, whereas antibody-mediated rejection would be more apparent in discordant combinations. The titer of preformed xenoantibodies and ease of inducing xenoantibodies were proposed to be able to provide an assessment of phylogenic diversity; hence, the designation of discordant and concordant combinations. Somewhat misleadingly, the patterns of discordant and concordant rejection have been used synonymously with disparate and closely related cross-species transplantation, respectively. Unfortunately, the definition is blurred by the variable susceptibility of different organs to antibody-mediated rejection. For instance, liver xenografts appear to be less susceptible to xenoantibody rejection compared with heart and kidney xenografts.[14] These findings have also been found in allografts that have been placed into a hostile, preformed antibody environment.[15]

In xenotransplantation, preformed antibodies occur naturally without the necessity of prior exposure to antigens from other species of animals. These antibodies are capable of mediating rejection, which may be hyperacute or may take place over a period of days to weeks. It is thought that these naturally occurring antibodies are the results of exposure to common environmental antigens, such as those composed by isoagglutinins of the ABO system. These antibodies react with various glycolipids and glycoproteins on the cell surface of the xenograft. The antigenic determinants that are targets of xenoantibody binding have been the focus of a great deal of research. It has been suggested that the primary cell surface epitopes for binding to xenoantibodies are carbohydrate in nature. One such candidate is the alpha 1 – 3 linkage of the subterminal and terminal galactose residues,[16–18] although other candidates have been identified.[19]

Generally, xenoantibodies are of the immunoglobulin M (IgM) class, but high titers of immunoglobulin G (IgG) can be induced by sensitization. In some discordant xenotransplant combinations, IgM and IgG can be shown to pre-exist in high titers, such as in the guinea pig – to-rat, the pig-to – rhesus monkey, and the pig-to-dog combinations. In concordant xenotransplant combinations, usually only low-titer IgM exists, such as in the hamster-to-rat, the fox-to-dog, and the rhesus monkey – to-baboon combinations. However, in both discordant and concordant combinations, sensitization after xenotransplantation generally results in an abrupt rise in the IgM titer followed shortly by an increase in the IgG titer.

The nature of the B-lymphocytes, which synthesize xenoantibodies, has been the subject of investigation, with hopes that identification of these cells may lead to specific therapies to eliminate them. It has been suggested that CD5+ B cells are the most likely candidates as the source of xenoantibody production.[20] In the rat, these cells can be identified in the spleen and are located in the splenic red pulp and in the marginal zone. Thus, the rationale for a role of splenectomy in xenotransplantation is to reduce the B cell load, which is thought to synthesize xenoantibodies.[21, 22]

Independent of the nature of immunoglobulin class of preformed antibody involved in triggering antibody-mediated rejection, the pathophysiology of the acute inflammatory response is similar. Preformed antibodies trigger inflammation and injury by their deposition on the endothelium of the vascularized graft. These antibodies, in turn, activate complement, which, in turn, activates a characteristic cascade of inflammatory, nonspecific mediators, such as recruitment of polymorphonuclear leukocytes, platelet adhesion, and degranulation followed by intravascular thrombosis. Complement activation can occur via the classic and alternative complement pathways (Fig 54 – 1). In the classic pathway, the C1q component of C1 is activated after binding to the Fc region of IgM and IgG. This, in turn, results in C1r and C1s activation and the generation of the C1qrs protease complex, in turn leading to C4 and C2 cleavage, producing the C3 convertase, C4b2a complex. C3 is then cleaved to produce the biologically active components C3a and C3b. In the alternative pathway, complement can be activated via immunoglobulins A and E and other nonimmunological factors, such as various polysaccharides and bacterial antigens. Activation of C3 occurs via nonspecific cleavage to generate C3b. The common, final pathway of complement activation is via C5 cleavage, which generates C5b, which, in turn, leads to the assembly of the C5b-C9 membrane attack complex (MAC). This MAC binds to the cell surface, resulting in a porous membrane that is susceptible to osmotic pressure, leading to either cell damage or cell death.

The importance of complement in the pathophysiology of antibody-mediated rejection is shown in studies in which complement is depleted. Cobra venom depletes the C3 and C5 components, resulting in paralysis of the complement system. Adachi and coworkers were able to obtain prolongation of discordant xenograft survival with the addition of cobra venom factor along with cyclosporine and an antiplatelet agent.[23] Other evidence of the importance of C5 in the process of xenograft hyperacute re-

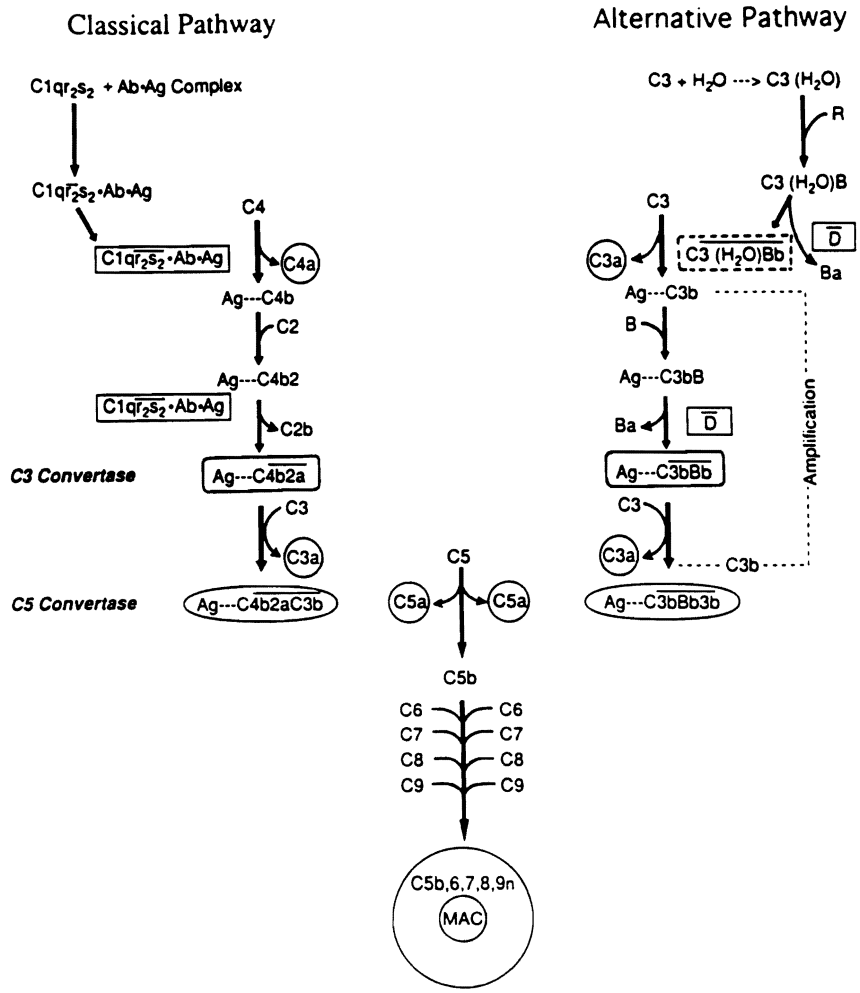

Figure 54-1 Classic and alternative pathways of complement activation leading to formation of the membrane attack complex (MAC).

jection is the ability of a sesquiterpene compound with anticomplement activity to prolong xenograft survival. K76 is thought to block the C5 step of complement activation and also accelerates the degradation of C5b.[24] Administration of 200 mg/kg to rats undergoing heterotopic guinea pig heart transplantation (discordant combination) resulted in marked prolongation of survival from 8 minutes to more than 8 hours.[25]

Cells express naturally occurring proteins (regulators of complement activation) on the cell surface, which help to modulate the effects of various complement-activated components. The molecules are thought to provide an intrinsic mechanism to limit the amplification of complement activation. Homologous restriction factor (CD59) and decay-accelerating factor (CD55) are two proteins that have been described as mediators of complement activation. CD59 is thought to act by inhibition of the insertion of C9 into MAC, thus aborting the terminal attack sequence of complement activation.[26] Decay-accelerating factors limit the generation of classic and alternative complement pathway convertases.[27] The importance of these modulators has been demonstrated by experiments that have enhanced expression of these proteins by gene transfection.[28] The activity of these complement modulators are thought to be species specific and help to explain the phenomenon of "homologous species restriction."[29] This phenomenon is most easily seen when the addition of homologous complement to susceptible target cells does not effectively cause lysis, whereas the addition of heterologous complement leads to effective cell lysis.

Cell damage also occurs by activation of other inflammatory pathways. Reactive oxygen metabolites, prostaglandins, and cytokines can be generated by the degradation products of complement activation. Polymorphonuclear leukocytes and macrophages are attracted to the site of inflammation as a result of the presence of the C5a fragment, which results in the release of lysosomal enzymes and resultant cell damage. C3b enhances adhesion of these cells to damaged cells and also enhances binding of platelets, which may lead to degranulation and release of vasoactive substances, such as serotonin and histamine, both increasing vascular permeability.

Thrombosis of the microvasculature is enhanced by the loss of membrane-associated heparan sulfate from the endothelial cell.[30] Heparan sulfate proteoglycan is present in the endothelial cell layer of normal vessels and helps to maintain a local anticoagulant environment by activation of antithrombin III, an inhibitor of thrombin generation. The release of tissue factors from injured cells promotes thrombosis.

The role of the cellular immune response in the destruction of xenografts has been difficult to determine mainly

because of the overwhelming rapid destruction of these grafts by naturally occurring xenoantibodies. Nevertheless, there is sufficient evidence that xenogeneic cells can elicit a strong cell-mediated response. Using discordant combination of human T cells to xenogeneic porcine stimulator cells, several investigators have shown that unprimed human T cells are capable of responding to porcine stimulators by proliferation and cytotoxicity.[31, 32] The characterization of the mechanisms of sensitization in vitro has not yet been clearly determined (ie, whether recipient antigen presenting cells require xenoantigen processing or whether xenoantigens can be presented directly by xenogeneic cells).

CURRENT XENOTRANSPLANTATION RESEARCH

The prospect of using animal organs for transplantation has been limited by the ability to control immune reactivity. The area that requires further investigation is the management of the antibody component of the immune response, which has been refractory to standard cyclosporine and steroid therapy.

Although a number of animal models have been developed for both discordant and concordant xenotransplantation, long-term successes have been limited for the most part to concordant combinations. The lack of high-titer preformed xenoantibodies has avoided the almost immediate destruction of discordant xenografts, which has been difficult to prevent or treat. Nevertheless, even in the discordant combinations, significant prolongation of xenograft survival has been reported when antibody depletion[33] or depletion of complement is used (see prior discussion).

Techniques to prevent or mitigate humoral allograft or xenograft rejection have been summarized elsewhere.[34] Of these approaches, prostaglandin therapy appears to have some potential with minimal side effects. Prostaglandin can mitigate the xenograft rejection in a number of experimental xenograft models.[35-37] There were early reports that prostaglandin would be effective against B cells.[38] The poorer prognosis of lymphocytotoxic crossmatch–positive liver allograft recipients was eliminated when prostaglandin was added to tacrolimus (FK506).[39] Although prostaglandin is weakly immunosuppressive,[40, 41] its unique effect in the xenograft system is via different targets than those of classic immunosuppression. Prostaglandins diffusely modify effectors of the inflammatory response, including cytokines.[42]

Although the duality of humoral and cellular mechanisms of xenograft rejection is generally accepted, it is also common knowledge that the antibody component is difficult to modulate. Consequently, additional strategies have been developed to minimize antibody-mediated rejection. Using a hamster-to-rat cardiac heterograft model, modest titers (1:16–1:32) of preformed heterospecific cytotoxic antibodies destroy the heart xenograft within 3 days in untreated rat recipients before there is histopathological evidence of lymphocyte infiltration. By itself, tacrolimus, which prevents T-cell activation and cytokine secretion, was able (at doses of 2 mg/kg/day) to prolong survival by only 1 day. Monotherapy with a number of antiproliferative drugs,[43-49] including those that suppress purine (RS61443) or pyrimidine (brequinar) synthesis, as well as the conventional anticancer drug cyclophosphamide could increase survival but did not permit consistent chronic survival. However, when some of these antiproliferative drugs were added to tacrolimus for the first 2 postoperative weeks, further survival under continued tacrolimus alone became routinely possible.[50]

After hamster-to-rat orthotopic liver xenotransplantation, the perioperative survival of the liver, with its well-known resistance to antibodies, was less dependent than the heart on the antimetabolite component of the combined drug therapy, but the results also were significantly improved with the antimetabolite drugs, including cyclophosphamide. Thus, it is clearly possible, with tacrolimus-based immunosuppression, to transplant heart and liver xenografts with consistent long-term survival of healthy recipients. These results are remarkable in the context of the information on this difficult model published in the literature.[51-55] Van Den Bogaerde et al[55] emphasized the separateness of the humoral and cellular mechanisms of xenograft rejection.

The therapeutic benefit of the adjuvant agents correlated with their ability to inhibit the antihamster antibody response postoperatively. Once the first 2 weeks had passed, treatment with antiproliferative agents was no longer necessary. Other circumstantial evidence supports the conclusion that the effectiveness of the adjuvant drugs is primarily by reducing the humoral antibody response. Flow cytometric studies of splenic B-cells after stimulation with xenograft tissue revealed that the addition of cyclophosphamide totally abrogated the blastic response of the CD5+ B cell, which is thought to synthesize heterograft-specific antibody.[21]

Because the spleen produces antidonor antibodies to xenotransplants, the effect of splenectomy combined with tacrolimus on heart and liver xenograft survival has also been studied. Using the hamster-to-rat model, cytotoxic antibody titers were markedly suppressed during the whole period of observation in both liver and heart xenotransplanted animals that received both tacrolimus and splenectomy. In contrast, untreated liver xenografts had cytotoxic antibody titers by day 7 (1:8192), whereas heart xenografts had a cytotoxic antibody titer of 1:256 on day 3. Thus, splenectomy may be an adjuvant in the strategy to control humoral rejection in clinical xenotransplantation. Splenectomy as an adjuvant immunosuppressant measure was introduced in 1962[56] and used extensively in the early days of transplantation,[57] especially for treating patients with preformed antibody states.[58]

The need to inhibit complement after liver xenotransplantation may be limited to a short period after implantation. It is clear that the principal source of complement is the liver.[59] With the ability to obtain long-term survival of liver xenografts in animal models, Valdivia et al have shown that the transition of complement from recipient to donor sources may have a long-term protective effect on the transplanted xenograft.[60] The phenomenon of homologous species restriction of complement activation may subsequently protect the xenograft from further complement-mediated damage.

SUMMARY OF THREE CLINICAL LIVER XENOGRAFT ATTEMPTS

Within an 8-month period, three attempts were made to transplant liver xenografts in the United States. The liver appeared to be a logical starting point because of the known relative resistance of the liver to antibody-mediated rejection[15] compared with the heart and kidney and also because of the lack of artificial support, such as dialysis or ventricular assist devices. Two baboon-to-human liver transplants were performed at the University of Pittsburgh, and one pig-to-human liver transplant was performed at Cedars-Sinai Medical Center in Los Angeles.

Pig-to-Human Liver Xenotransplantation

In the case of the pig-to-human liver transplant, the transplant group at Cedars-Sinai, headed by Leonard Makowka, had prepared a protocol for the temporary support of a failing human liver ("bridge to transplant").[61] The protocol was designed to treat patients with acute deterioration of liver function, necessitating emergency support and transplantation, and was written in response to the death of a patient before the availability of a human liver for transplantation. The Institutional Review Board, along with the Ethics Committee, approved the protocol, which was designed to support a failing human liver until a permanent human liver replacement could be found. The single patient who underwent transplantation at that center was given a pig liver only after exhaustive efforts had been made to find a human liver and after the condition of the patient deteriorated. The patient's family gave consent after detailed discussions.

Pigs can grow to weigh more than 300 pounds. The size difference can be minimized by selecting a donor organ that is slightly larger than that of a human. Selection of the donor was based on size and was obtained from a breeding farm after a period of quarantine. The pig was sedated and given inhalation anesthesia during liver procurement.

The recipient of a pig liver was a 26-year-old woman with accelerated liver failure from autoimmune hepatitis who progressed into grade 3–4 coma. Most humans have high titers of preformed antibodies against pig tissue, which is not related to ingestional exposure to pork.[62] The patient was treated to remove preformed anti-pig antibodies using a combination of plasmapheresis and specific antibody removal by passage of her blood through a set of pig kidneys. The immunosuppression was based on cyclosporine, cyclophosphamide, prostaglandin E_1, and azathioprine. The liver was placed in a heterotopic position. The liver appeared to function for 20 hours, as demonstrated by an initial decrease in the serum lactate level and the presence of bile. However, the ammonia level did not fall and by 20 hours after transplantation several clinical parameters worsened, and the patient died from brain death 26 hours after transplantation. The liver appeared to have undergone extensive infarction from hyperacute rejection in spite of the lowering of the antibody titer using plasmapheresis and pig kidney perfusion.[63] These findings are consistent with those described by Chantrel et al using a pig-to-primate model of liver and kidney immunoabsorption, in which rapid reaccumulation of xenoreactive antibodies was observed after initial immunoabsorption.[64]

Baboon-to-Human Liver Xenotransplantation

The rationale for the baboon-to-human liver transplant was for treatment of end-stage liver disease caused by chronic active hepatitis B. Hepatitis B is the leading cause of liver disease in the world, affecting more than 250 million people. It has been shown that human liver transplantation into patients with hepatitis B (especially with positive viral replication markers, eg, hepatitis B e antigen or hepatitis B DNA positivity) is associated with a high rate of reinfection of the new liver and an accelerated cirrhosis after transplantation.[65, 66] In many parts of the world this disease is considered a contraindication to human liver transplantation. Experimental agents have been used with only limited success in the prevention of recurrent hepatitis B. Baboons have been thought to be resistant to the development of chronic active hepatitis B (Landlord RE, Southwestern Primate Facility, San Antonio, TX, unpublished data, 1993). Thus, the principal benefit to the patients enrolled in this trial was the possibility that the baboon liver would not be reinfected and that the stigmata of chronic liver disease would be reversed. The transplant was, therefore, considered a permanent replacement for the failing human liver.

Nonhuman primates offer a number of advantages in the study of liver transplantation and as donors for xenotransplantation. Unlike the canine or porcine liver, the liver anatomy of nonhuman primates is similar to that of humans. Among the higher order primates, similarities exist between the major histocompatibility complex and the cellular markers found in the immune system. The blood groups are similar to the A and B blood types, although O blood types are quite rare.[67]

Primates are composed of two suborders: Prosimii and Anthropoidea. Prosimian primates resemble squirrels or rats more than true monkeys. The Anthropoidea suborder can be further subdivided into five different families: New World monkeys, Old World monkeys, lesser apes, great apes, and humans. From an investigational standpoint, the most frequently used species are the Old World monkeys. This family includes rhesus monkeys (*Macaca mulatta*), cynomologus monkeys (*M. fascicularis*), and baboons (*Papio cynocephalus*). Great apes include the chimpanzee (*Pan troglodytes*) and gorillas (*Gorilla gorilla*) but are not used in large numbers as donors because of their endangered classification, although they approximate humans in genetic similarity and size more than do lesser apes.

The liver anatomy in 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 primates, the central lobe fuses with the right and left lateral lobes. The quadrate lobe is much more narrow in nonhuman primates than in humans, and the caudate lobe may encircle the circumference of the inferior vena cava. The ligamentous attachments are similar to those described in humans. The blood supply to the

liver is similar in the Anthropoidea, with a great variation in the arterial blood supply. The portal venous system is essentially identical in the higher order primates. The hepatic venous drainage is similar to that of humans, with small, short hepatic venous tributaries draining the right and central lobes and 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 arterial supply is usually from a branch from the right hepatic artery. The cystic duct joins the common hepatic duct a variable distance to form the common bile duct before emptying into the duodenum.

Baboons are physically smaller than humans. The maximum weight of an adult male baboon is about 65 to 70 pounds. Unlike the heart, a small liver can rapidly grow to accommodate the function expected by the size of the recipient,[68] although the largest baboons were selected for the clinical trials in liver xenotransplantation. A closed colony of bred baboons was screened to match blood groups with the recipients, and all known infectious agents were screened, including serologies for retroviruses (simian immunodeficiency virus [SIV], simian retrovirus [SRV], simian T lymphotrophic virus [STLV], human immunodeficiency virus [HIV] types 1 and 2, foamy virus, and human T cell lymphotropic virus), DNA viruses, cytomegalovirus (CMV), Epstein-Barr virus, herpes virus (SA8 and varicella-zoster virus), hepatitis viruses (hepatitis A, hepatitis B, and hepatitis C viruses), and other potentially transmittable agents (such as Marburg virus, encephalomyocarditis virus, lymphocytic choriomeningitis virus, hemorrhagic fever virus, tuberculosis, and toxoplasma).[69] The animals were thoroughly examined and a complete biochemical analysis and compatibility by histocompatibility testing of the donors made. A number of suitable donors were screened to select the donor with the lowest reactivity by lymphocytotoxicity studies, using the recipients' serum. Once selected, the animals were quarantined. No other preparation was required, and the animals were sedated and given inhalation anesthesia during procurement. A certified veterinarian was present to ensure compliance to animal handling standards. A complete autopsy was performed after the liver had been taken out for transplantation. The protocol for this trial was designed with the input of a panel of extramural transplant specialists, the Institutional Review Board, a panel of infectious disease consultants, and intramural specialists in histocompatibility, hepatology, immunology, and transplantation. After a detailed discussion of informed consent, both patients and their families consented to the procedure.

The following summarizes the clinical courses of the two baboon-to-human liver transplant patients.[70] The first patient was a 35-year-old man who received a number of blood transfusions as a result of previous trauma. In 1989 he also underwent splenectomy after a motor vehicle accident. That year, the patient was also found to be HIV positive, but his CD4 lymphocyte count was normal as were the in vitro mitogen responses. His blood group was type A. The second patient was a 62-year-old man in whom the cause of hepatitis B was not determined. His blood group was type B. He had not had any previous abdominal operations. In both patients, the principal complications of chronic hepatitis B were poorly controlled edema, fatigue, ascites, encephalopathy, and gastrointestinal bleeding. Because of the nature of their liver disease, these patients were not considered candidates for human liver transplantation, but because of the severity of complications, xenotransplantation was considered an experimental option.

In both patients receiving a baboon liver, slight technical modifications were made to the standard orthotopic liver transplant technique, using the piggyback technique described for smaller donor liver venous outflow reconstruction.[71] In the first patient, a 600-g liver was removed from the baboon and implanted; the second patient received a donor liver weighing 450 g. Because of the considerable size discrepancy, the portal vein of the first donor was anastomosed to the recipient's left portal vein branch. In the second patient, an end-to-end portal vein anastomosis was done. In the first patient, the donor's celiac axis was anastomosed end to end onto the recipient's common hepatic artery. In the second patient, the arterial reconstruction required a donor carotid artery interposition graft to the recipient's supraceliac aorta. In both patients, a Roux-en-Y choledochojejunostomy was used to reconstruct the biliary drainage system.

The cold ischemia time was 80 minutes in the first patient and 231 minutes in the second patient. Both patients experienced uneventful intraoperative courses after reperfusion of the baboon liver, and the clinical impression was one of immediate function of both xenografts. Immunosuppression used a combination of tacrolimus, steroids, prostaglandin E_1, and cyclophosphamide. Of the various antimetabolites that were shown to provide a synergistic immunosuppressive effect in experimental xenotransplant models, cyclophosphamide was chosen because of its relative availability and its proven usefulness in allotransplantation.[72] Except for slightly higher doses of tacrolimus in the first 2 posttransplant weeks, the doses of tacrolimus, steroids, and prostaglandin were within standard therapeutic dosages. Cyclophosphamide administration was started 2 days before transplantation and continued for a total of 56 days and 10 days (patient 1 and patient 2, respectively) at dosages ranging from 0.07 to 10.6 mg/kg/day.

The first patient awoke promptly after transplantation and was extubated after 17 hours. He was placed on an oral diet on posttransplant day 5. The liver function tests returned toward normal by the second posttransplant week, and the levels of transaminases returned to normal within the first week (Fig 54-2). The second patient never regained a level of consciousness that permitted weaning from the ventilator. In addition, the quality of the liver function in the second patient was suboptimal, with persistent hyperbilirubinemia during the entire postoperative course (Fig 54-3). Nevertheless, in both patients, there was evidence of adequately functioning liver mass, such as normalization of the coagulation with normal prothrombin time, correction of the hyperammonemia, normal arterial ketone body ratio (a manifestation of hepatic energy stores), and clearance of serum lactate.

A number of liver biopsies were obtained throughout the posttransplant course.[73] The pathological changes in both patients revealed an early element of mild antibody

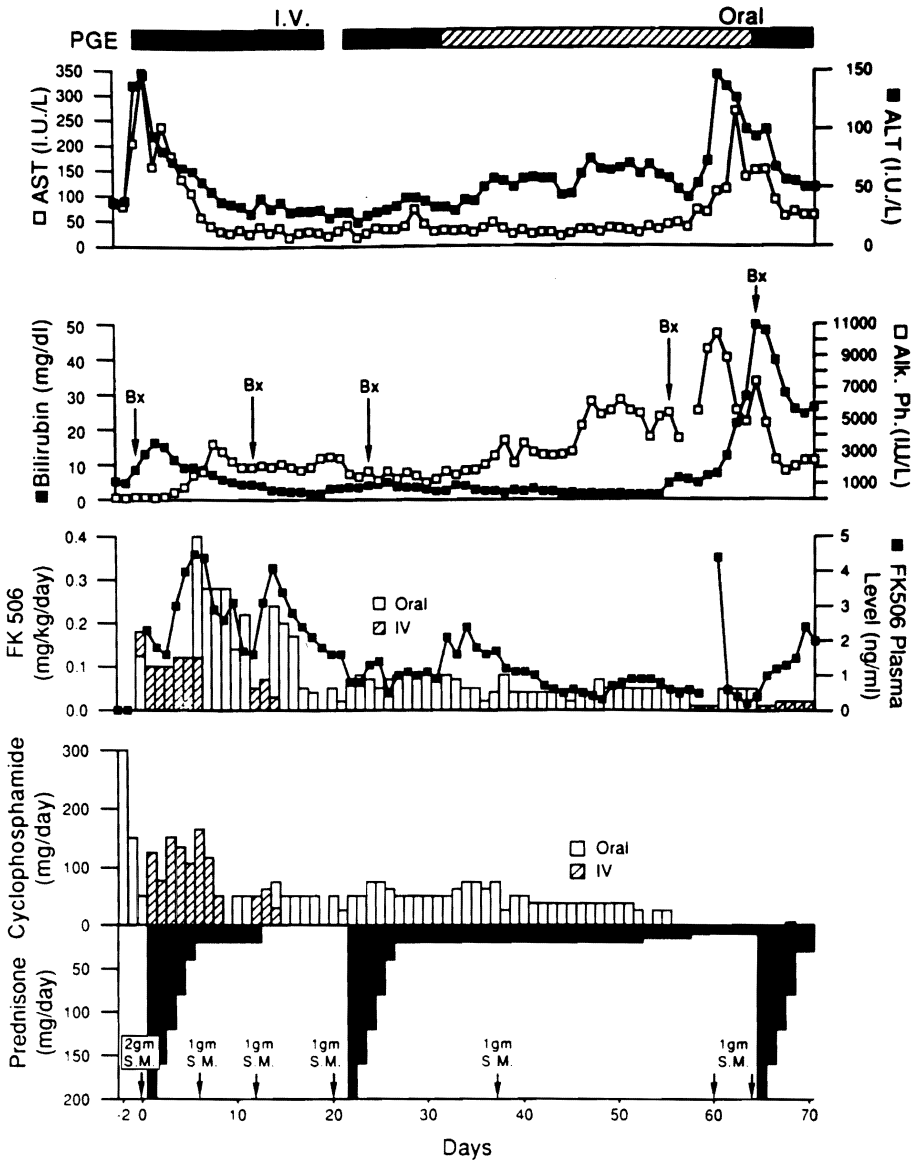

Figure 54-2 Clinical course of first baboon-to-human liver transplant patient. (From Starzl TE, Fung J, Tzakis A, et al. Baboon-to-human liver transplantation. Lancet 341(8837):65-71, © by The Lancet Ltd. 1993.)

attack without substantial cell-mediated rejection. The earliest postperfusion biopsies (4 hours after perfusion) revealed some mild antibody-mediated insult. Immunofluorescence revealed binding of immunoglobulin with complement deposition; however, neither endothelial injury nor platelet aggregation was seen. Polymorphonuclear cell infiltration and natural killer cells were seen in these early biopsies. In the first patient, a biopsy taken on day 12 revealed Kupffer cell hypertrophy, mild centrilobular hepatocyte swelling, and cholestasis with a mild mononuclear portal and perivenular infiltrate. This infiltrate was predominantly T cell and was consistent with a mild cellular rejection with minimal antibody injury. Later biopsies were remarkably free from rejection, either antibody or cellular. Mild cholestasis was noted in some of these later biopsies. In the first patient, the final antemortem biopsy was taken on posttransplant day 64 and revealed obvious bile infarcts with mural necrosis of segments of the septal bile ducts. In the second patient, an intraoperative biopsy taken on posttransplant day 4 revealed indirect evidence of antibody-mediated rejection, and a subsequent splenectomy was performed.

Complications in both patients occurred, some possibly related to technical issues and others resulting from the immunosuppression used to prevent rejection. There were a number of infectious complications related to both technical causes and immunosuppression. Many of these infectious agents are typical of the microorganisms seen in allotransplantation (eg, *Staphylococcus, Candida,* CMV, *Enterococcus,* and *Aspergillus*). In the first patient, the contribution of the positivity of both the recipient and donor to CMV to the subsequent CMV infection is not known. Attempts to determine the origin of the CMV, either human or baboon, failed because of the inability to culture the CMV from clinical samples in sufficient quantity to subtype. The susceptibility of both baboon and human CMV to ganciclovir diminishes the impact of the origin of this virus.

Other complications included renal failure and dialysis dependence as well as a number of iatrogenic complica-

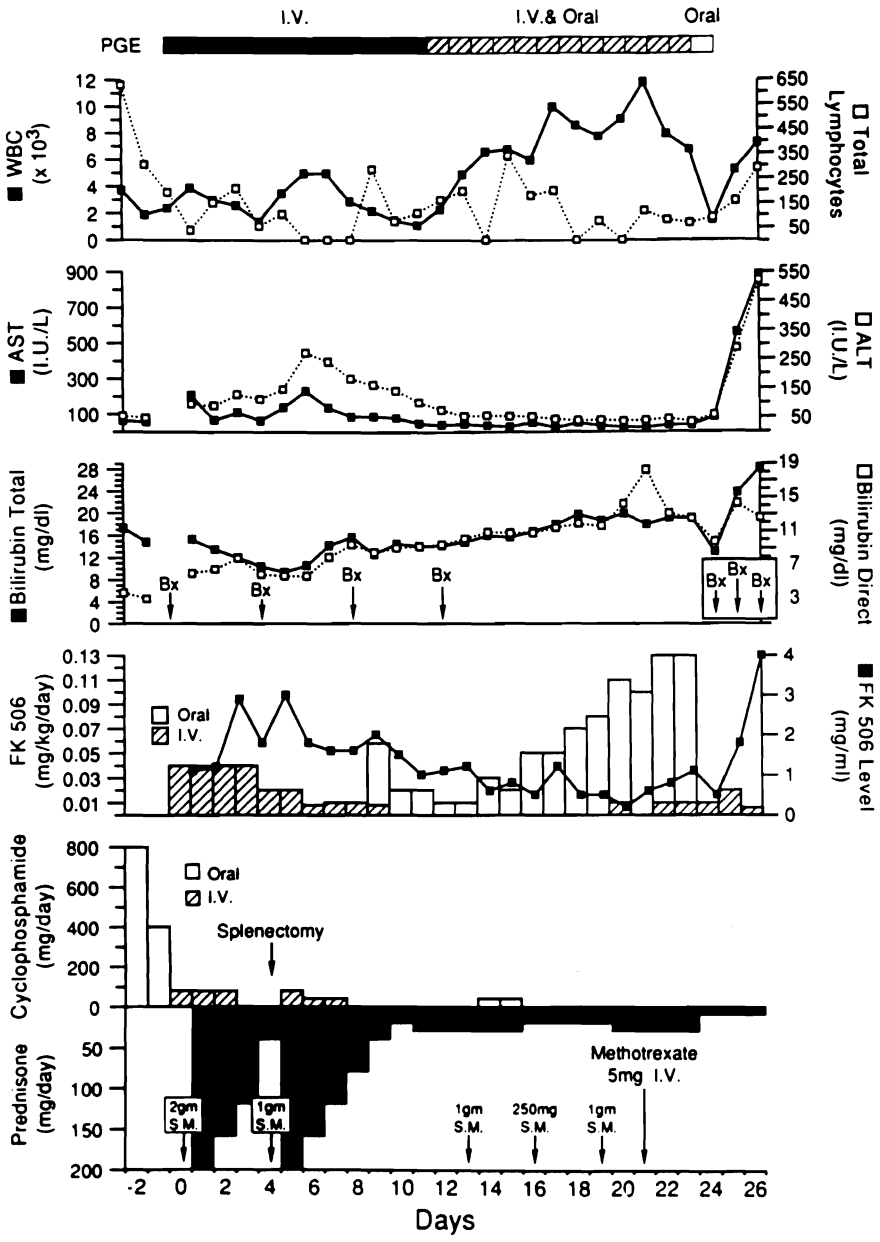

Figure 54-3 Clinical course of second baboon-to-human liver transplant patient. (From Starzl TE, Tzakis A, Fung JJ, et al. Human liver xenotransplantation. Xeno 1:4-7, 1993.)

tions. The cause of the renal failure was probably multifactorial, with contributions from drug toxicity (including tacrolimus, amphotericin, vanocomycin, ganciclovir, and trimethoprim-sulfa-methoxazole), and rejection. The first patient was able to be taken off hemodialysis for a short period but required resumption for uremic gastritis. The second patient did not recover any significant renal function during the 26 days after xenotransplantation.

The causes of death in both patients were also multifactorial. In the first patient, a rise in both the alkaline phosphatase and total bilirubin levels prompted a percutaneous transhepatic cholangiogram on posttransplant day 61. Within an hour after the procedure, the patient became hypotensive, febrile, and coagulopathic. This patient was intubated and was stabilized and improved over the last 5 days of his life. A positive culture for *Aspergillus flavus* was noted and full-dose amphotericin administration was started. On the last day of his life, the patient was being weaned from the ventilator, with stable coagulation factors, and was hemodynamically stable. The patient suddenly deteriorated neurologically, and a computed tomographic scan of the head revealed a massive subarachnoid bleed. At autopsy, the cause of death was determined to be subarachnoid hemorrhage resulting from angioinvasive aspergillosis. In this patient, two foci of aspergillosis were found in the lung, with focal dissemination to the kidney and brain. The bile ducts of the liver were slightly dilated, and numerous bile infarcts were detected. The biliary anastomosis appeared somewhat stenotic. Although the patient was in kidney failure, the kidneys appeared intact, and there was no evidence of immune complex deposition in the kidney.

The second patient died on posttransplant day 27 from complications of peritonitis. It is likely that the high doses

of corticosteroids, used to control early immunological damage, were responsible for poor tissue healing, leading to a leak from the enteric anastomosis.

Both baboon livers were shown to grow rapidly from baseline values of 600 g and 450 g to more than 1555 g and 1741 g, respectively. Radiographically, there was evidence of accommodation of the vasculature to the growth in liver size. Finally, the baboon liver did affect the protein profile of both patients. Liver-specific proteins could be shown to be of baboon origin by serum protein electrophoresis. In both patients, the recipients acquired the same coagulation profile as the baboon and retained a normal prothrombin time and coagulation profile.[74] No evidence of adverse effects of these proteins could be found, such as immune-mediated kidney injury. Total complement levels were depleted for 1 to 2 weeks after liver xenotransplantation, similar to that reported in liver allotransplantation across a positive lymphocytotoxic crossmatch.[75]

Serial determinations of hepatitis B surface antigen failed to find serological recurrence of hepatitis B, and immunostaining for hepatitis B virus in the liver biopsies also confirmed lack of reinfection. Because 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 assays using polymerase chain reaction could not detect hepatitis B DNA in the transplanted liver.[76]

In summary, baboon livers transplanted into two humans, using four agent immunosuppression, were able to sustain life for 70 and 26 days, respectively. The livers appeared to function during this period, although liver function was compromised during the later posttransplant course. In these xenotransplant cases, it is conceivable that the unrecognized biliary stasis syndrome was a manifestation of an unrecognized technical limitation or, more likely, an atypical manifestation of rejection. The contrasting posttransplant courses of the two patients may be partially explained by differences from an immunological standpoint (splenectomy and HIV infection in the first patient) and patient selection (the second patient was elderly and more critically ill at the time of transplantation).

ETHICAL ISSUES

A number of ethical concerns regarding the field of xenotransplantation have been raised, including issues of informed consent, safety, and animal rights concerns. Many issues surrounding the field of xenotransplantation are extensions of debates regarding broader issues of health care, biomedical research, organ transplantation, and human experimentation. We do not address the debate on animal rights because the arguments for using animals for biomedical research are far beyond the scope of this discussion.

The principal concerns that have attracted the attention of the scientific community and the medical ethicists deal with informed consent, safety of the experiment, and timeliness or appropriateness of the procedure. The issue of timeliness or appropriateness of xenotransplantation is the least controversial. The need for finding a solution to the organ shortage is real. The number of human donors in the United States has not increased appreciably in the past 5 years. For only 25% of the potential brain dead donors is consent given for donation. The effectiveness of seatbelt laws and drunk-driving laws has diminished the "normal" pool of head trauma patients. The drop in this category of donors has been compensated for by expanding the criteria acceptable for donors, including expanded age criteria and the inclusion of medical conditions in the donor that were once excluded. Unless there is a major reform in public attitudes toward donation or expanded legislation to entice or require donation, it is unlikely that the current organ shortage will be rectified. Given an increase in the understanding of the mechanisms of antibody-mediated rejection and the development of new agents for immunosuppression, cautious trials will help to identify the areas in which further research is warranted. The need to continue research into clinical xenotransplantation is highlighted in this environment.

Informed consent is a matter of open discussions between the patient (and family) and his or her physician. Sufficient information must be supplied by the physician, without an air of assumption, in order for the patient to weigh the risks and benefits of the course that he or she is about to embark on. To the extent that regulatory committees view the informed consent process, they must ensure that the patient agrees by his or her own volition, without coercion, either overtly or subliminally, to participate, with the physicians not directly imparting their opinions or making the decision for the patient. It is difficult to determine the contribution of desperation to decision-making, especially when the alternative is death. In this light, regulatory forces may take on a surrogate role in preventing decisions based solely on "grasping at straws." In both of the baboon-to-human liver xenotransplant attempts, the informed consent period encompassed lengthy discussions with the patients, their families, the transplant team, and third-party observers over a 7-day period.

The scenario of unleashing a "doomsday" infectious agent on the human species has been forwarded by some.[77] The possibility of transmission of infectious agents after xenotransplantation is finite, but it is the "unknown" agent that imparts caution in these trials.[78] The risk of infection when using either discordant or concordant donors should also be considered in the overall balance of risk-benefit discussions. On one hand, primate donors have the advantage of genetic similarity (and therefore, potential compatibility) and less risk of immunological loss. On the other hand, pig donors are more easily raised and are not sentient animals. Nevertheless, the possibility of disease transmission from porcine donors exists.[79] Technological advances and better screening tools are likely to identify donors that may harbor latent infections or allow design of genetically engineered animals that may not be as susceptible to rejection.

Xenotransplantation faces criticism that is strongly reminiscent of that leveled against human-to-human transplantation in the late 1960s and early 1970s. Yet with persistence the field of human-to-human transplantation has proven to be highly successful. This success was the result of stepwise increases in understanding the biology of rejection, improvements in drug management, and experience. It is possible that xenotransplantation may not be

universally successful until further technological advances occur, yet cautious exploration of xenotransplantation appears warranted to identify those areas that will require further study.[80]

CONCLUSIONS

The trial and error of the cited pioneer trials 3 decades ago defined the human barrier to several species used. Success was tantalizingly close with the chimpanzee, baboon, and rhesus monkey (in that order). Although the chimpanzee has been shown to be a biologically superior donor (less genetic diversity) in the early xenografting trials, the threat of extinction of chimpanzees precludes further such trials. In contrast, the baboon is easily raised in captivity and is not an endangered species. The difficulty of heterotransplantation will vary with different organs. Because it is relatively resistant to antibody-mediated rejection, the liver is the organ for which there is the greatest chance of success.

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