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In a patient with life-threatening organ failure, "bridging" with a porcine organ graft until an allograft becomes available would be one approach to initiating exploration of clinical xenotransplantation. Whether temporary support with a porcine xenograft will sensitize the human recipient to a subsequent allograft, resulting in possible humoral or accelerated cellular rejection, remains uncertain. Few studies have addressed the question of whether allotransplantation can be safely carried out after a xenograft 'bridge'.

There are some studies in rodents, but their relevance to the pig-to-human model is questionable. There has also been a study in dogs, but again the relevance of the data to clinical xenotransplantation is doubtful. Organ xenotransplantation from foxes into dogs increased the risk of rejection of a secondary allograft.[1] Primary cardiac allografts in dogs were rejected in 18 days. When fox hearts were transplanted into dogs, they were rejected in a mean of 10 days, whereas subsequent dog cardiac allografts were rejected in a mean of 5 days. Five out of 9 dogs lost the allograft in an accelerated manner, being rejected within 2–48 hours, with histologic features of humoral rejection. In this model, therefore, prior xenografting would appear to be detrimental to subsequent allografting. The literature that is available on "bridging" in primates has been reviewed. This brief review includes work in concordant and discordant nonhuman primate experimental studies (Table 1) and reports of clinical experience with pig-to-human ex vivo liver hemoperfusion.

Allotransplantation After a Concordant Xenograft in Nonhuman Primates

In the first study reported in the literature, prior xenotransplantation of hearts from African green monkeys in five baboons (with survival from 5–65 days) did not result in hyperacute rejection of subsequent allografts (with survival from 10– >198 days).[2] The immunosuppressive therapy given to the recipients varied, and early rejection tended to occur in the first three baboons, in which therapy was less than optimal. In the last two baboons, long-term allograft survival (>5 months) was achieved, with no features of rejection at the time of graft explantation. However, even in one of these, there was evidence of accelerated cellular rejection on endomyocardial biopsy within the first 2 weeks, although this responded well to increased immunosuppressive therapy. With adequate immunosuppression, therefore, this study suggested that allografting could be successful after rejection of a concordant xenograft.

In a second study, in a series of six African green monkey-to-baboon auxiliary liver transplants,[3] graft survival was from 10 to >120 days, with graft loss usually from a cell-mediated response. Only one of these baboons subsequently developed alloantibodies when the sera were tested (from 10 to 48 days after xenotransplantation) against a lymphocyte panel from five or six baboons, and in this case the serum was cytotoxic to only two of the five targets. Lymphocytotoxicity was negative in the remaining five baboons. One of these baboons subsequently received a cardiac allograft (while the auxiliary monkey liver was still functioning three months after it had been transplanted). The cardiac allograft functioned well for 30 days, at which time the baboon was euthanized. At this time, features of moderate-to-severe acute cellular rejection were present in the allograft, but there was no evidence of antibody-mediated rejection.

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Table 1 **EXPERIENCE WITH SECONDARY ALLOGRAFTING AFTER PRIMARY CONCORDANT OR DISCORDANT XENOGRAFTING IN BABOONS**

RECIPIENT	PRIMARY DONOR (ORGAN)	GRAFT SURVIVAL	SECONDARY DONOR (ORGAN)	GRAFT SURVIVAL	REF.
Baboon	AGM (heart)	5–65 days	Baboon (heart)	10 ≥198 days	2
Baboon	AGM (liver)	10 ≥120 days	Baboon (heart)	>30 days	3
Baboon	Cynomolgus monkey (heart)	>14 days	Baboon (heart)	>56 days	4
Baboon	Pig (liver, heart, blood)	1 hour–6 days	Baboon (liver, heart)	6– >62 days	3

AGM = African green monkey

Whether temporary support with a porcine xenograft will sensitize the human recipient to a subsequent allograft, resulting in possible humoral or accelerated cellular rejection, remains uncertain.

In a third study, hearts from cynomolgus monkeys were transplanted into four immunosuppressed (cyclosporine, azathioprine, steroids) baboons for two weeks.[4] After this time, two of the four recipient baboons were found to have developed alloreactive cytotoxic antibodies directed against the MHC class II-like antigens expressed by more than 50% of lymphocytes obtained from of a panel of 12 baboons. One of these "sensitized" baboons received a heart from an allo-donor to which it had developed cytotoxic antibodies. However, no obvious detrimental effect on the rapidity or severity of allograft rejection was noted. The allograft functioned throughout the 8 week observation period, and the histological features on endomyocardial biopsy were no different from those seen in the other three transplanted hearts, which also functioned well and showed moderate, potentially reversible (grade 3A) acute cellular rejection. The primary xenograft, therefore, did not appear to induce humoral or cell-mediated immune responses that jeopardized the survival of the secondary baboon cardiac allograft.

Allotransplantation After a Discordant Xenograft in Nonhuman Primates

The literature is particularly sparse in discordant models, as there appears to be only one study addressing this important issue.[3] After six baboons had been sensitized by pig tissue (following either auxiliary liver or heart xenotransplantation or the transfusion of pig blood), no alloantibodies could be detected in any of the baboon sera against a panel of lymphocytes from five donor baboons. However, there had been a delay of from 2–12 months between exposure to the xenograft and testing for the presence of alloantibodies. When secondary auxiliary liver allografting was performed in three of these baboons, one liver suffered some ischemic injury (not thought to be immunologically-induced) and the other two functioned well without histopathological features of hyperacute, humoral, or accelerated cellular rejection, although some acute cellular rejection occurred. Initial xenotransplantation with a pig organ or cells, therefore, did not appear to lead to sensitization to a subsequent baboon organ.

Clinical Observations

Recently, two patients have undergone liver allotransplantation following "bridging" by ex vivo hemoperfusion of porcine livers.[5] The livers were obtained from pigs genetically modified to express human complement-regulatory proteins. In both patients, this relatively transient exposure to xenoantigens, including, of course, swine leukocyte antigens (SLA), followed by liver allotransplantation and long-term immunosuppressive therapy, failed to induce anti-HLA antibodies. Levels of antibodies directed against Galα1-3Gal (Gal) epitopes rose markedly after the ex vivo pig liver hemoperfusion, indicating sensitization, and persisted at a moderate level in both patients. One patient developed early acute cellular rejection in the allograft, requiring antilymphocyte antibody therapy. In the second patient, a rejection episode responded well to corticosteroid therapy.

Bridging with a bioartificial liver (BAL), which incorporated porcine hepatocytes, has also been followed by successful liver allotransplantation.[6] Immediately after the first BAL treatment, anti-pig IgG and IgM levels decreased by 20%. In those patients who underwent two or more BAL treatments, however, strong anti-pig IgG and IgM responses were observed by day 10. No differences in the levels of anti-pig antibody were observed between those patients who subsequently received pharmacologic immunosuppressive therapy (required after liver allotransplantation) and those who did not receive such therapy.

Comment

The unpredictable availability of human cadaveric donor organs makes bridging with a xenograft an appealing approach to initiating a clinical trial of xenotransplantation. Such an approach would enable experience of xenotransplantation to be gained without committing the patient long-term to a xenografted organ. The patient's life would be supported, for example, by an implanted porcine liver or, possibly, heart, until a suitable human organ became available. This approach would be carried out in the full knowledge that it would not contribute to a reduction in the demand for human organs (which is the ultimate goal of xenotransplantation) and, indeed, would actually increase the demand by maintaining a patient alive until allografting was possible. Nevertheless, it would provide a "learning curve" for those involved with the development of xenotransplantation. Answers to many important questions might be obtained. For example, does the presence in humans of natural preformed antibodies to non-Gal antigens (rarely seen in nonhuman primates) increase the risk of humoral rejection of a pig organ in a human? Are the immunomodulatory regimens tested and proven to be successful (even if only for a period of weeks or months) in the nonhuman primate equally successful in humans? Although the literature is sparse, particularly with regard to prior xenotransplantation with pig organs, the reported experimental and clinical studies suggest that prior exposure to xenoantigens may not prove a major problem by sensitizing to alloantigens. Cross-reactive sensitization may further be avoided if the recipient receives an anti-CD154 monoclonal antibody during the period of exposure to the xenoantigens, as our own group has demonstrated in the pig-to-baboon model that this prevents an induced antibody response.[7]

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Nevertheless, so few studies have been performed specifically to explore this potential problem that it is premature to come to a definite conclusion. Three of the studies reviewed above were made in concordant, rather than discordant, models, which may not reflect the pig-to-human situation. Furthermore, in the only discordant study, there was a considerable delay between the development of rejection of the xenograft (and excision of the organ) and in vitro testing for the presence of alloantibodies and/or transplantation of the allograft. The outcome may possibly have been different if testing and allografting had been performed at the time of xenograft rejection. However, these experimental data, and the few clinical observations that have been made, encourage us to believe that function of an allograft may not be jeopardized by the presence of cross-reactive antibodies that have developed as a result of a bridging xenotransplant.

References

1. Hammer C, Schutz A, Pratschke J, et al. Bridging to transplant: Allogeneic heart transplantation after xenografting. **J Heart Lung Transplant. 1992; 11:182-188**.
2. Alonso de Begona J, Gundry S, Kawauchi M, et al. Assessment of baboon lymphocyte subsets and activity in cardiac xenobridging to allotransplantation. **Transplant Proc 1992; 24:453-454**.
3. Ye Y, Luo Y, Kobayashi T, et al. Secondary organ allografting after a primary "bridging" xenotransplant. **Transplantation 1995; 60:19-22**.
4. Michler RE, Shah AS, Itescu S, et al. The influence of concordant xenografts on the humoral and cell-mediated immune responses to subsequent allografts in primates. **J Thorac Cardiovasc Surg. 1996; 112:1002-1009**.
5. Levy MF, Crippin J, Sutton S, et al. Liver allotransplantation after extracorporeal hepatic support with transgenic (hCD55/hCD59) porcine livers: Clinical results and lack of pig-to-human transmission of the porcine endogenous retrovirus. **Transplantation 2000; 69:272-280**.
6. Baquerizo A, Mhoyan A, Kearns-Jonker M, et al. Characterization of human xenoreactive antibodies in liver failure patients exposed to pig hepatocytes after bioartificial liver treatment: an ex vivo model of pig to human xenotransplantation. **Transplantation 1999; 67:5-18**.
7. Buhler L, Awwad M, Basker M, et al. High-dose pig hematopoietic cell transplantation combined with CD40 ligand blockade in baboons prevents an induced anti-pig humoral response. **Transplantation 2000; 69:2296-2304**.