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The Biological Basis of and Strategies for Clinical Xenotransplantation

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INTRODUCTION

We will begin with a brief summary of our previously reported experience with clinical xenotransplantation (Starzl et al. 1964a, 1969b, 1989c, 1993d, 1994e). However, the purpose of the exercise is to delineate the fundamental issues that must be resolved before such procedures can be tried again, and to identify strategies that may bring this objective within reach, as well as those which are apt to be futile.

The choice of models for research in xenotransplantation predetermines the mechanisms of xenograft destruction. With one kind of species combination (for example, rat- or human-to-mouse; wolf-to-dog; sheep-to-goat; subhuman primate-to-human), transplanted tissues or whole organs are subject to cellular rejection after a delay of several days in much the same way as allografts, but with a more prominent humoral component. With the contrasting "forbidden" combinations (i.e. guinea pig-to-rat, pig-to-dog, or pig-to-human), organs are hyperacutely rejected by a process that is usually associated with preformed xenospecific antigraft antibodies.

The common assumption that the two kinds of outcome mirror unrelated pathways has added to the mystique of xenotransplantation. This misconception has been perpetuated by terminologies such as "concordant versus discordant" (Calne 1970) that have several indefensible implications, as discussed recently by Makowka & Cramer (1994) and Leventhal & Matas (1994): exclusivity of the two

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mechanisms, a determinant role of phylogenetic distance, and uniqueness of the xenospecific humoral reaction relative to that seen with allografts. Recent "discoveries" about the events of humoral xenograft rejection have tended to reinforce these stereotypic conclusions, while largely ignoring an earlier literature leading to the quite different conclusion that xenograft rejection, including the hyperacute variety, is merely an extreme expression of mechanisms that also can afflict allografts (Starzl et al. 1968, 1970).

BABOON-TO-HUMAN XENOTRANSPLANTATION

It was the perception of a similarity to allotransplantation that prompted the baboon-to-human renal xenotransplantation trials at the University of Colorado in 1963 before extensive research in whole organ xenotransplantation had been done (Starzl et al. 1964a). The only known examples of hyperacute allograft rejection had been in recipients of ABO-mismatched kidneys (Starzl et al. 1964f, 1964g), causing us to underestimate the potential risk of immediate graft loss. Twenty-nine years later, the fact that the baboon kidney xenografts had not hyperacutely rejected, plus the availability of vastly improved immunosuppression, prompted a trial of baboon liver transplantation for dying patients who were disqualified by medical criteria for hepatic allotransplant candidacy.

The kidney trials (1963–1964)

The 6 patients given baboon kidneys in late 1963 and January 1964 underwent simultaneous bilateral nephrectomy and splenectomy, and were treated postoperatively with azathioprine and prednisone. Earlier in that year, Reemtsma et al. (1964) had paved the way with encouraging results using chimpanzee kidney xenografts, one of which functioned for 9 months. Although a handful of chimpanzee xenografts were used subsequently (Cortesini et al. 1970), including a heart (Hardy et al. 1964) and 3 livers (Starzl et al. 1969b, 1989c), social opposition to using such an anthropomorphic and endangered species already had served notice that extensive further trials would be unacceptable. We then learned from Dr. Claude Hitchcock of Minneapolis that he had secretly performed a similar operation even before Reemtsma's first case, using a baboon kidney which had not been hyperacutely rejected. As reported later by Hitchcock et al. (1964), this first xenograft of modern times was lost at 4 days from an arterial thrombosis which was suspected to have a technical etiology.

Confirming Hitchcock's observation that baboon kidneys escaped hyperacute rejection, the 6 Colorado xenografts functioned for 6 to 60 days (Starzl et al. 1964a, 1989c). At the end, they developed fierce cellular rejection (Porter 1964). However, the key histopathologic finding was occlusive endothelialitis of the graft vessels that had choked off much of the arterial supply. The consequent distal

ischemia explained a patchy gangrene of the xenografts, interspersed between islands of still functioning parenchyma (Porter 1964). The extensive arterial lesions were similar to but more acute than those previously reported in allografts by Porter (1963). The same kinds of gross and histopathologic findings were reported more than 20 years later by Bailey et al. (1985) after baboon cardiac xenotransplantation under a cyclosporine-based immunosuppressive regimen (the Baby Fae case).

Recipient titers of preexisting anti-donor leukocyte agglutinins declined irregularly throughout the residence of the xenografts in these recipients (Starzl et al. 1964a, Kirkpatrick & Wilson 1964). Three of the 6 patients received ABO-mismatched kidneys and fared no worse than the others. The changes of the preexisting ABO isoagglutinin titers in all patients were random. This was not surprising because ABO antigens are weakly expressed in baboon tissues and red blood cells.

The conclusion from this experience was that xenospecific humoral rejection had been responsible for the failures (Starzl et al. 1964a, Porter 1964, Kirkpatrick & Wilson 1964, Starzl 1964h). Because the futility of proceeding without an effective means of antibody control was obvious, a moratorium on further trials was self-imposed that lasted for 29 years.

The liver trials

The patients – In June 1992, and January 1993, 2 patients with end-stage chronic active hepatitis caused by B virus (HBV) had their livers replaced with baboon organs, with survival of 70 and 26 days (Starzl et al. 1993d, 1994e). The conventional lymphocytotoxic crossmatch of the recipient sera with their donor lymphocytes was positive initially but negative after dithiothreitol (DTT) treatment, indicating that the antibodies were predominantly IgM (Starzl et al. 1993d, 1994e). Postoperatively, even the unaltered crossmatches became negative. Both patients had donors of their own blood type, A to A in Case 1 and B to B in Case 2. The recipients were immune-competent preoperatively with *in vitro* testing, although Patient 1 had symptomatic HIV infection, and had undergone post-traumatic splenectomy 3 years previously. This patient was half the age (35 years) of Patient 2 and far less frail, still semi-conscious, and not yet on ventilator support. Patient 2 underwent splenectomy 4 days post-transplantation.

Immunosuppression – Treatment was with FK 506, prednisone, cyclophosphamide, and prostaglandin E_1 (PGE_1). There were specific reasons for the addition of 2 drugs to the conventional FK 506-prednisone combination originally developed for allografts. Prostaglandin E_1 (PGE_1) in combination with high induction doses of prednisone had been shown not only to eliminate most of the risk to liver allografts posed by a positive lymphocytotoxic crossmatch (Takaya

et al. 1992a) but also to reduce the acute risk of FK 506 nephrotoxicity (Takaya et al. 1992a, 1993). These properties have made PGE_1 a routine constituent of our present-day 3-drug immunosuppressive cocktail for all liver allograft recipients.

Although PGs are inherently but only weakly immunosuppressive (Strom & Carpenter 1983, Quagliata et al. 1972), they have been reported to mitigate humoral rejection by tempering the cytokine-mediated inflammatory response (Shaw 1985, Rapaport & Dodge 1982) after hamster-to-rat (Shaw 1985, Kakita et al. 1975a), cat-to-dog (Mundy 1980), and pig-to-dog (Makowka et al. 1987) xenotransplantation.

The 4th drug, cyclophosphamide, is one of several agents that inhibit DNA synthesis, suppress antibody responses by preventing B-cell clonal expansion, and prolong the survival in rats of hamster xenografts (Hasan et al. 1992, Murase et al. 1993). It was chosen in preference to several other agents with the same general "antiproliferative" mechanism (Murase et al. 1993) because it was an accepted drug in the formulary, and had been substituted for azathioprine as a conventional immunosuppressant for allotransplantation in the era preceding cyclosporine (Starzl et al. 1971).

Causes of failure – There was no evidence postoperatively of recurrent HBV infection, nor any overt histopathologic findings of cellular rejection in multiple liver biopsies and at autopsy. Infectious complications were the immediate causes of death: ruptured mycotic intracerebral aneurysm caused by aspergillus in Case 1, and peritonitis secondary to a leak at the Roux-y biliary reconstruction in Case 2. However, the underlying cause of death in both cases was suboptimal xenograft function which was not satisfactorily explained at the time. In retrospect (see later), it was evident that the cause had been a slow-motion form of humoral rejection.

The patient, who lived for 70 days, was jaundice-free for most of his survival. However, his serum alkaline phosphatase became elevated from the 2nd week onward, suggesting partial biliary obstruction. At autopsy, the entire biliary tree was filled with inspissated bile, and most of the biliary ducts, which had become bile lakes, were denuded of epithelium. The 2nd patient never woke from his preexisting coma, and never cleared bilirubin. At autopsy, his xenograft also was filled with inspissated bile.

The manifold physiologic manifestations of hepatic dysfunction included the inability of either xenograft to maintain a postoperative serum albumin above 2 g%. Both patients developed renal failure which may have had a component of the hepatorenal syndrome. The tendency to ascribe the postoperative graft dysfunction to rejection resulted in overimmunosuppression. The dichotomy of response in which cellular immunity was essentially eliminated but with inadequate control of humoral immunity (Starzl et al. 1993d, 1994e) is the core dilemma of xenotransplantation.

The 30-year period bracketed by the baboon kidney xenotransplantations at one end and the liver trials at the other was almost contemporaneous with the modern era of transplantation. We will attempt to show here that all of the advances made in between in allotransplantation, and more significantly the failure to achieve some objectives, are directly applicable to xenotransplantation. To illustrate the point, we will ask and attempt to answer a series of specific questions.

DOES XENOTRANSPLANTATION POSE UNIQUE TECHNICAL PROBLEMS?

None of the 6 kidney xenografts failed because of technical complications. However, the occurrence of lethal biliary tract problems in both baboon xenograft recipients mandated a close review of the operative procedures. Although the techniques, which were adapted from hepatic allotransplantation (Starzl et al. 1993d, 1994e), seemed satisfactory, the body weights of the baboon donors were only 40% of those of the recipients, necessitating the so-called piggyback operation which leaves the recipient vena cava intact. The livers regenerated up to optimal volume for recipient size in both cases. The biopsies had the typical findings of regeneration, with multitudes of proliferating hepatocytes and duct cells, with almost no infiltrating immunocytes.

Shiraishi et al. (1994) have reported that the use of small to large liver allografts (and by inference xenografts) introduces an increased antigenicity factor. In Shiraishi's experiments, the increased MHC Class II expression that is independently associated with liver regeneration (Jonjic et al. 1987) appeared to be responsible for more severe cellular and humoral rejections than seen with size-matched liver transplantation in the same rat strains. (Shiraishi et al. 1994).

However, this adverse factor was not thought to be great enough to account for the dysfunction of the xenografts, particularly because it could be easily overridden with a brief course of immunosuppression (Starzl et al. 1994i).

DO XENOGRAFTS IMPOSE METABOLIC INCOMPATIBILITIES?

Having the same cholestatic problem twice without clear evidence of mechanical obstruction raised the possibility that the baboon liver produced a lithogenic bile in the human environment. The global dysfunction of the hepatic xenografts militated against such a specific non-surgical explanation. However, the more generic question of metabolic incompatibility has not been laid to rest. After hepatic replacement, liver allografts continue to produce donor-phenotype proteins and other synthetic products, allowing hepatic replacement to be used to correct numerous liver-based inborn errors of metabolism (Starzl et al. 1989j). Because the same retention of donor specificity occurs after successful xenotransplantation (Starzl et al. 1993d, 1994e, 1993k, Valdivia et al. 1993a), the consequence of suc-

cessfully engrafting a liver xenograft could be equivalent to transplanting an inborn error of metabolism. That this can occur with allografts has been demonstrated by the accidental transplantation of a coagulation disorder (Dzik et al. 1987) and by the evidence that cholesterol patterns change to those of the donor following liver replacement (Kraft et al. 1989).

During the survival of the 2 human liver recipients, metabolic "baboonization" included albumin, C_3 complement component, properdin, and other moieties involved either in classical metabolic processes, immune reactions, or blood coagulation (Starzl et al. 1993d, 1993k). The fall of the patient's serum uric acid postoperatively to the nearly undetectable level that is normal for the baboon was a particularly dramatic demonstration of the creation by the hepatic xenograft of its own chemical environment (Starzl 1993k). Although no specific harm could be identified from these changes, both patients required unusual quantities of red cell and platelet infusions postoperatively which were suspected (but not proven) to represent GvH reactions caused by antibodies elaborated from the disseminated baboon nonparenchymal cells (NPCs) (see later).

The most complete metabolic studies after liver transplantation (Valdivia et al. 1993a) have been in the hamster→rat rodent interspecies combination in which the phylogenetic distance by paleontolologic and genetic evidence has been estimated at 15 to 40 million years (Hartenberger et al. 1985). In this model, the donor/recipient clotting tests are disparate, the most striking difference being that Protein C which is plentiful in normal hamsters is undetectable in rats. The coagulation profile of the rat recipients of orthotopic hamster livers quickly was hamsterized, without adverse consequences of bleeding or clotting (Valdivia et al. 1993a). The change to donor-specific products of hepatic synthesis of albumin and other moieties also appeared to be compatible with healthy survival.

However, the clinical signs of donor-determined metabolic aberrations may require years for development. After Rhesus monkey to baboon transplantation, Gridelli et al. (1993) have reported a B_{12} deficiency which, although correctable, was postulated to have a metabolic basis. If there are serious metabolic consequences of using disparate species donors, xenotransplantation will be limited to organs such as the kidney and heart with less complex functions. In addition, alien complement synthesized by the hepatocytes and the antihost antibodies produced by the NPCs of all organs have immunologic consequences (Ramsey et al. 1984).

IS XENOGRAFT ACCEPTANCE ASSOCIATED WITH CHIMERISM?

A characteristic cycle of crisis and recovery was defined 30 years ago in human renal allograft recipients in which the successive events were rejection, its reversal with intensified treatment, and the later ability to wean immunosuppression (Starzl et al. 1963). When studied 3 decades later, a cohort of these original

patients who still bore their original allografts were demonstrated to have ubiquitous low level donor leukocyte chimerism (Starzl et al. 1992l, 1993m). With this discovery and its confirmation in recipients of other kinds of organs (most prominently the liver) (Starzl et al. 1993n, 1992o, 1993p, 1993q), the events following transplantation could be reconstructed in terms of a bi-directional cell traffic and a reciprocal immune transaction between the graft and host (Demetris et al. 1993, Qian et al. 1994). These consisted of a graft-versus-host reaction in addition to the conventional host-versus-graft (rejection) response (Starzl et al. 1992, Demetris et al. 1994) – each eventually cancelling the effect of the other in successful cases. The result was that the graft as well as the recipient became genetic composites.

Because the emigrant donor cells ("passenger leukocytes") from the graft were multilineage and derived originally from the bone marrow, the organ transplantation was in effect a mini-bone marrow transplantation. We now are supplementing these cells in unconditioned organ allograft recipients by giving them unaltered donor bone marrow at the time of organ transplantation. Postoperative treatment is with conventional immunosuppression. All of these allograft recipients (n=36) are well and all have chimerism, which is 1000-fold or greater than that which occurs spontaneously (Fontes et al. 1994a). Eventually, drug weaning and discontinuance is planned.

Striking microchimerism was demonstrated throughout life and at autopsy in both of our baboon liver recipients (Starzl et al. 1993d, 1994e). The first recipient, whose survival was 70 days, was given only a liver. Thus, the baboon DNA found by PCR in all tissues retrieved at autopsy represented donor leukocytes that had migrated from the liver. Patient 2, who had been infused with 3×10^8/kg baboon bone marrow cells after the liver xenograft was revascularized had similar mixed xenogeneic chimerism until death.

If xenotransplantation is to be successful, it seems evident that the persistence of the double cell population is both inevitable and obligatory (Fig. 1). In chronically surviving rat recipients of hamster hearts and even more dramatically in those given liver xenografts, chimerism was regularly seen within the grafts (Valdivia et al. 1993b) and systemically (Valdivia et al. 1993c). The discontinuance of immunosuppression after 100 days in these animals was followed by weeks or months of survival without further treatment before the slow onset of rejection (Valdivia et al. 1993b).

There are still vast gaps in this new paradigm of graft acceptance, but some large ones already have been filled by chimerism studies in the mouse (Lu et al. 1994). These have shown how potentially tolerogenic precursor dendritic cells and possibly other components of the multilineage migratory cells may perpetuate themselves after export from organ allografts. The concept of this transmutation in both the transplanted organ and the recipient has many potential implications in xenotransplantation. By knowing how graft acceptance occurs, the possibility has been opened of mimicking the circumstances for its accomplishment.

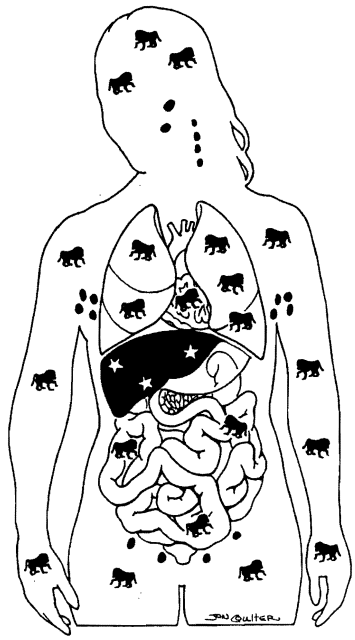

Figure 1. The double cell leukocyte population in the transplanted whole organ as well as the recipient which is an obligatory condition for acceptance of either allografts or xenografts. This recently defined paradigm (Starzl. et al. 1992, 1993a, 1993b) provides a basis for innovative therapeutic strategies of xenotransplantation.

DOES "HEPATIC TOLEROGENICITY" APPLY TO XENOGRAFTS?

Cellular immunity

The immunologic advantages of hepatic allografts *and xenografts* include a greater ease of inducing their acceptance with a limited course of immunosuppression (Starzl et al. 1965, 1969s, Murase et al. 1990a, Valdivia et al. 1991) or, in some swine (Garnier et al. 1965, Peacock & Terblanche 1967, Calne et al. 1967) or rat (Zimmerman et al. 1979, Kamada et al. 1981, Murase et al. 1990b) and virtually all mouse (Qian et al. 1994) strain combinations, with no treatment at all. Another property is the liver's unusual ability to induce a state of immunologic unresponsiveness to other tissues and organs of the same donor strain (Kamada et al. 1981, Calne et al. 1969, Valdivia et al. 1993c). We have explained these qualities by the heavy endowment of the liver relative to other organs (Starzl et al. 1992l, 1993n, 1993q) with potentially migratory cells of multilineage phenotypes.

Humoral immunity

The relative resistance of the transplanted liver to antigraft antibodies that cause hyperacute allograft *and xenograft* rejection (Kamada et al. 1981, Starzl et al.

1974, Houssin et al. 1985, Furuya et al. 1992), as well as the ability of the liver to shield other organs from humoral rejection (Fung et al. 1988, Flye et al. 1990) is of special interest for xenotransplantation (Valdivia et al. 1993c). The shielding from humoral rejections is MHC-restricted for allografts (Fung et al. 1988, Flye et al. 1990). However, in the hamster→rat xenograft model the protection covers all third-party hamsters (Valdivia et al. 1993c), presumably because of the inbred nature of this species. The explanation (next section) for the liver's antibody resistance is central to strategies that may allow successful clinical xenotransplantation.

Complement

Because the liver is the primary source of complement synthesis (Alper et al. 1969, Wolpl et al. 1985), allogeneic or xenogeneic liver transplantation changes the recipient complement system to that of the donor (Valdivia et al. 1993a, 1993c). However, *in vitro* culture studies of human and rodent mononuclear phagocytes (Colten 1976, Cole & Cotten 1988) showing synthesis of complement components means that there also are extrahepatic complement sources. Consequently, the recipient's complement conversion after liver xenotransplantation to that of the donor species is by definition incomplete. Nevertheless, the species complement change has been proved by direct experimentation to be the mechanism by which the hamster liver xenograft can shield all third-party hamster organs from humoral rejection (Valdivia et al. 1994). In these experiments, hamster hearts were transplanted into stable rat recipients of hamster liver xenografts. Then, hyperimmune antihamster sera from different species containing activated and inactivated complement was injected intravenously (Valdivia et al. 1994). The results are summarized in Figs. 2 and 3.

In essence, the introduction of the active rat complement contained in hyperimmune antihamster rat serum caused prompt hyperacute rejection within a few minutes of a secondarily engrafted hamster heart that otherwise would have been accepted under the protective umbrella of the previously engrafted hamster liver, and also caused rejection of the liver itself within a few hours. These lethal events were completely prevented by the simple expedient of decomplementing the serum by heating at 56°C for 30 min. This demonstration of the species specificity and efficacy of the complement system was confirmed by transplanting other species organs and by injecting other species antisera. The results were congruent with exhaustive *in vitro* assays including complement-dependent cytotoxicity (Valdivia et al. 1994).

It remains to be determined in genetically controlled models if the trans-species protection from humoral rejection (as well as from cellular rejection) endowed by the new complement environment in the hamster→rat model has no MHC restriction or, more likely, if these findings reflect the intensive inbreeding and minimal genetic diversity in the hamster, as we have suggested (Valdivia et al. 1993c).

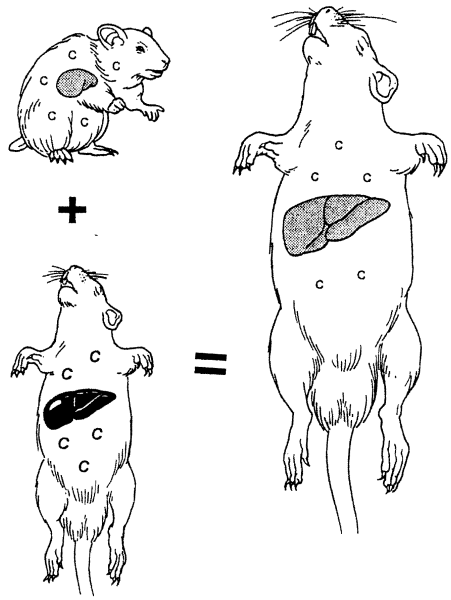

Figure 2. Demonstration by Valdivia et al. (1994) that the complement environment of a xenograft recipient becomes that of the donor species. In these experiments, the dominant complement of rat recipients of hamster livers become hamster-specific within a few days, a change that was permanent. See text for further explanation.

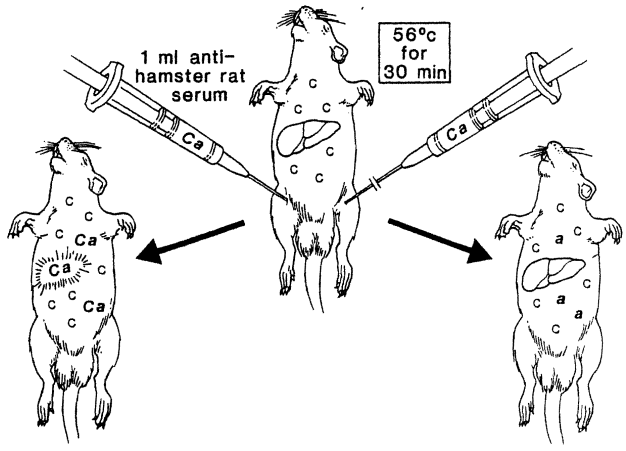

Figure 3. Second stage experiments of Valdivia et al. (1994) following the engraftment shown in Fig. 2, showing species restriction of complement activation. Rats bearing long-surviving hamster livers hyperacutely rejected these xenografts when injected with rat-hamster serum, but not if the serum was first decomplemented by heating.

MHC restriction of complement in the context of hyperacute allograft rejection has been reported by Fung et al. (1988) and in more detail by Flye et al. (1990) in a clinical allograft setting. They showed that hypersensitized patients given liver allografts could then receive kidneys without the expected complication of humoral renal rejection. What must be accomplished for survival of either a liver allograft or xenograft (or organs to which they extend protection) is survival and function of the new liver long enough to allow the complement transition to begin. "Self rescue" by this means is lost too quickly in difficult xenograft models for this to occur.

These findings plus the concept of chimerism as the mechanism of graft acceptance have suggested strategies for xenograft organ preparation that may avoid complement activation (see final section of this article).

CAN XENOGRAFT CELLULAR REJECTION BE CONTROLLED?

Although the hepatic tolerogenicity factor may have been contributory, the improved immunosuppression of 1993 compared to that used in 1963 was thought to be the principal explanation for the virtual freedom of the hepatic baboon xenografts from the severe cellular rejection that was seen in earlier renal and cardiac cases. However, there is very limited information about what might be accomplished with species combinations in which the organs are hyperacutely rejected before drugs such as cyclosporine and FK 506 can act. This "experimental artefact" which precludes direct study of cellular rejection is not different from the plight of the hypersensitized human recipient for whom the provision of T-cell directed immunosuppression is a hollow gesture.

The magnitude of this barrier in xenograft models correlates less well with phylogenetic distance than with the preexisting antigraft antibody status. For example, successful protection of skin xenografts from humans and other species transplanted to the distantly related mouse has been reported with ALS (Lance & Medawar 1968) or even without treatment (Steinmuller 1970). The unusual ease of xenotransplantation to mice from several species can be explained more easily by this animal's well-known low complement activity against these species (Gorer 1958, Haensch et al. 1981) than by the distance of the donor on the evolutionary scale.

It is particularly noteworthy that xenogeneic cells can be engrafted more easily than complex tissues and organs (Hullett et al. 1987, Ricordi et al. 1987, Faiqui et al. 1991, Michejda et al. 1992, Srour et al. 1993). This has been illustrated by a recently reported experiment (Fontes et al. 1994b) in which 2 baboons preconditioned with 7.5 Gy total lymphoid irradiation were given $6x10^8$/kg unaltered human bone marrow cells, and provided with no subsequent treatment. There was PCR evidence of human DNA in the blood of 1 animal for more than 6 months, and permanently in the other. GvHD did not occur in either. Yet, when

the baboons were sacrificed at 18 months, donor DNA was found widely distributed in the tissues of both animals. In their bone marrow, colonies of burst-forming-unit erythroid (BFU-E), granulocyte (CFU-GM), and macrophage, and mega-karyocytes (CFU-GEMM) tested positive by PCR for donor human DNA. This evidence of extensive engraftment was confirmed by the identification of human cells in the liver and other tissues by staining with human-specific monoclonal antibodies (Fontes et al. 1994b).

In a more extreme well-documented example, xenoengraftment of porcine beta cells in humans was the only apparent explanation for the detection of C-peptide many months after the infusion of pig pancreatic islets into diabetic patients (Korsgren et al. 1992). This outcome contrasted with the hyperacute rejection by humans of pig hearts within a few minutes (Czaplicki et al. 1992) and of livers within a few hours (Eiseman 1966, Norman et al. 1966, Abouna et al. 1970, Makowka et al. 1993). The reason for the difference with cells versus organs appears to be that the prime target of xenograft rejection is the vascular system. Thus, a more meaningful assessment of cellular xenogenicity and the ability to control it undoubtedly is obtained from cell transplantation studies, and perhaps most discriminately with *in vitro* analysis.

In vitro studies have been done, but almost no one has been willing to accept the results at face value. For example, Auchincloss (1994), who demonstrated multiple defects in mouse T-cell response to primate stimulators (Moses et al. 1989), has suggested that there may be alternative undefined cellular mechanisms (such as NK cells). The instinctive reluctance to believe that cellular rejection of disparate xenografts is actually less vigorous than of allografts could be diminished (although not eliminated) by bone marrow xenotransplantation experiments in which the occurrence of GvHD is generally conceded to be dependent on T-cell reactivity.

Aside from the human→baboon experiments cited above, information about the consequences of bone marrow xenotransplantation in so-called concordant species combinations has been almost exclusively from rat→mouse or mouse→rat models. However, GvHD has now been produced in rats given hamster bone marrow following conditioning with 9.5 and 10.5 Gy total body irradiation (Patijn et al. 1994). These experiments proved the competence of xenogeneic T-cell reactions, but the vastly more interesting observation was the ease with which the GvHD could be controlled with FK 506 (unpublished data, Luis Valdivia).

Further information is badly needed about GvHD after engraftment (if this proves to be possible) with more difficult combinations and especially those in which strong antibody barriers preclude survival of whole organs for more than a few minutes. The critical question is if xenogeneic bone marrow from a disparate species can be engrafted and proliferate in the recipient without producing GvHD or with GvHD that can be readily controlled. Intriguing evidence that this might actually be possible was summarized by Simonsen (1962), but not pursued be-

cause the objective of the experiments was to study GvHD, not to develop models of engraftment in which the experimental end points were invisible.

Aside from their intrinsic interest, the bone marrow experiments of Patijn et al. (1994) have allowed more complete interpretation of the previous research on hamster→rat whole organ transplantation. The value of this species combination for xenotransplant experimentation was first reported for hearts by Barker & Billingham (1971) and Kakita et al. (1975b) and extended subsequently to orthotopic liver (Murase et al. 1993, Valdivia et al. 1987, Yamaguchi et al. 1990) and kidney models (Miyazawa et al. submitted). The events and mechanisms of hamster heart, kidney, and liver rejection in rat recipients have been extensively described (Valdivia et al. 1987, Yamaguchi et al. 1990, Miyazawa et al. submitted, Knechtle et al. 1987a, Monden et al. 1989, Van Den Bogaerde et al. 1991, Cramer et al. 1992, Langer et al. 1993) with emphasis on the preformed low level hamster-specific xenoantibodies. These antibodies have been demonstrated with *in vitro* cytotoxic assays using hamster lymphocytes or endothelial cells as targets and with an indirect immunofluorescence assay showing xenospecific immunoglobulin binding to tissues. Despite the ominous presence of these antibodies, hyperacute rejection of the heart, kidney, and liver does not occur. However, rapid B-cell activation and xenospecific antibody production causes pure humoral rejection of the heart and kidney in 3 days. The liver with its well-known resistance to antibody rejection (Murase et al. 1993, Valdivia et al. 1991) is destroyed in 7 days by a combination of humoral and cellular rejection.

The duality of the immune response, with a strong but briefly delayed antibody component, makes it easy to understand why the most potent T-cell directed agents, cyclosporine and FK 506, do not prolong the survival of hamster kidneys or hearts when used alone to treat the rat recipients, but become effective when they are combined with a long list of agents (or with splenectomy) that suppress the antibody response (Hasan et al. 1992, Murase et al. 1993, Valdivia et al. 1987, Yamaguchi et al. 1990, Miyazawa et al. submitted, Knechtle et al. 1987a, Monden et al. 1989, Van Den Bogaerde et al. 1991, Cramer et al. 1992, Langer et al. 1993). Murase et al. (1993), who provided the most complete studies of this kind, summarized by saying *"by breaking down the antibody barrier...it has been possible with FK 506 to transplant hearts and livers in the moderately difficult hamster to rat xenotransplantation model as easily as in many allogeneic strain combinations and more easily than in some"*. The list of these "adjuvant" drugs has become too long to review here but some which also have potent anti-T cell activity have been touted as primary drugs for xenotransplantation. The greatest claims have been for leflunomide (Xioa et al. 1994, Kemp et al. in press) and Brequinar (Makowka & Cramer 1994, Cramer et al. 1992).

Although these drugs all prolong xenograft survival, the rate of immunologically mediated complications of the xenografts of survivors is high despite provision of chronic T-cell directed immunosuppression. This has been particularly

well illustrated by the high rate of late bile duct and ureteral problems in liver and kidney xenografts. These organs usually have histopathologic evidence of inflammatory reactions but contain few or no lymphocytes. We believe that the abnormalities reflect ongoing humoral more than uncontrolled cellular rejection.

CAN HUMORAL REJECTION BE CONTROLLED?

This brings us squarely to humoral rejection, the central issue of xenotransplantation, and also the principal unresolved problem of allotransplantation. Because humoral rejection, whether of allografts or xenografts, has been the *cul de sac* of transplantation immunology for more than 30 years, it is important to have a precise idea of how our understanding of this complication evolved.

What does the term mean?

With ABO incompatibility – Hyperacute rejection was first observed more than 30 years ago when ABO-mismatched renal allografts were transplanted into patients who had preformed antigraft ABO isoagglutinins (Starzl et al. 1964f, 1964g). After the kidneys were lost on the operating table, arteriograms of the infarcted organs showed nonfilling of the small vessels, which correlated histopathologically with widespread thrombotic occlusion of the microvasculature. The conclusion was that the disasters had been caused by high-affinity isoagglutinins in the recipient sera, which had bound with A or B antigens in the graft vessels and parenchymal cells. This conclusion was supported by rapid changes in recipient isoagglutinin titers, as well as by the previous documentation by Szulman (1960) and by Hogman (1959) that the ABO antigens were widely distributed throughout human tissues and organs. The guidelines formulated from this experience (Starzl et al. 1964f, 1964g) were designed to avoid such antibody confrontations (Table I). The ABO rules also apply to heart
Starzl et al. 1987), liver (Starzl et al. 1987, Gordon et al. 1986, Demetris et al. 1988), and other kinds of organ transplantation.

However, not all ABO-mismatched organs met the same fate, and in fact the longest continuously functioning renal allograft in the world (Starzl et al. 1990) is a B^+ kidney donated to a 38-year-old A^+ male recipient by his younger sister on January 31, 1963. This variability of response was explained by Rapaport et al (1968) in one of his classical histocompatibility studies in which human volunteers were sensitized with purified A and B antigens. This caused increased titers of isoagglutinin. Accelerated or hyperacute (white graft) rejection of subsequently transplanted ABO-incompatible skin grafts completed the circle of evidence indicating the antibodies which in high titers presented an obstacle no less formidable than in the most severe xenograft models. However, it eventually was realized that the hyperacute rejection was only associated with, not dependent on, the anti-

TABLE I

O to non-O*	Safe
Rh− to Rh+	Safe
Rh+ to Rh−	Relatively safe
A to non-A	Dangerous
B to non-B	Dangerous
AB to non-AB	Dangerous

*O is universal donor, AB is universal recipient. However, in cases of ABO-compatible but not identical transplantation (O→A, B, or AB; A→AB; B→AB), this reverse violation of the ABO barrier is safe for the graft but it can subject the recipient to mirror-image complications in which donor immunocytes elaborate antibodies directed against the host. These usually are manifested by hemolytic or thrombocytopenic syndromes. This "humoral GvHD" in liver recipients (Ramsey et al. 1984) provided early clues to the generic paradigm of allograft acceptance via chimerism shown in Fig. 1 Starzl et al. (1992, 1993). This form of GvHD, of which the ABO system provided a particularly visible example, has not been well studied in xenotransplantation models but is certain to be important.

bodies and that it was more accurately described as a complement activation syndrome (see further on).

With non-ABO antibodies – Less than a year after the ABO disasters of 1963, the same outcome was reported by Terasaki et al. (1965) in a kidney recipient whose sera contained preformed antigraft lymphocytotoxic antibodies. The association of these antibodies with the hyperacute rejection led directly to the lymphocytotoxic crossmatch test. Terasaki's observations were promptly confirmed and extended by Kissmeyer-Nielsen et al. (1966), and others (Williams et al. 1986, Patel & Terasaki 1969). It was shown in animals as well as humans that antibodies, clotting factors, and formed blood elements were rapidly cleared by the grafts (Starzl et al. 1990, Simpson et al. 1970, Giles et al. 1970, Boehmig et al. 1971). Local fibrinolysis from the renal vein also was a consistent finding, and in exceptional cases, there were systemic coagulopathies with disseminated intravascular coagulation (DIC) (Starzl et al. 1970, Myburgh et al. 1969).

Additional non-HLA antibodies such as anti-vascular endothelial cell antibodies also have been associated with hyperacute or accelerated rejection (Cerilli et al. 1985, Brasile et al. 1985). The vulnerability of extrarenal organs including the antibody-resistant liver to this kind of rejection was ultimately recognized experimentally (Knechtle et al. 1987b, Gubernatis et al. 1987, Merion & Colletti 1990) and clinically (Takaya et al. 1992b). However, as with ABO incompatibility, many recipients with preformed antibodies failed to conform to expectations and did not reject their allografts.

Without detectable antibodies – The cause and effect relation of preformed antigraft antibodies and hyperacute rejection was universally accepted by 1969 when a seam

developed in the dogma. This was caused by the observation of hyperacute rejection in a small number of carefully studied human recipients who had no detectable antibodies (Starzl et al. 1968). Although the antibody-free cases were few in number relative to examples associated with antigraft antibodies, they necessitated redefinition of hyperacute rejection as a complement activation syndrome related to the Arthus reaction, inverse anaphylaxis, and the generalized Swarzman reaction (Starzl et al. 1968, 1970). Each of these classical complement-activation syndromes, characterized by multiple secondary inflammatory and coagulation events, is most commonly initiated by interaction of antibody and antigen, but they also can be independent of antibodies. In the transplantation context, this was the distinction *between the classical pathway of complement activation in which the first steps are antibody-dependent, versus the alternative pathway which does not require an antibody trigger or the participation of complement components C1, 2, and 4.*

Slow-motion hyperacute rejection – The exclusion of an allograft or xenograft from the circulation by hyperacute rejection is nature's life-raft. If blood flow is maintained to a kidney that is the cause of complement activation, clearance may continue of antibodies, formed blood elements (especially platelets), and clotting factors with an explosive systemic coagulopathy characterized by fibrinolysis (Starzl et al. 1968, 1970, Myburgh et al. 1969, Weber 1989). Emergency removal of the graft may be the only means of relieving the crisis (Starzl et al. 1970, Myburgh et al. 1969, Starzl et al. 1979t, 1979u). Even if this can be avoided, renal allografts that pass through such a storm have a degraded prognosis. Early or late failure was the rule in a group of hypersensitized patients whose serologically detectable antibodies and immunoglobulins of all classes were reduced by thoracic duct fistula for 30 to 56 days – a procedure that dramatically reduces humoral as well as cellular immune reactivity (Starzl et al. 1979t, Gowans 1959, Machleder & Paulus 1978, Niblack et al. 1975). Although hyperacute rejection of the allografts was prevented in 2/3 of the cases, all of the organs were lost in the next few months to combined humoral and cellular rejection (Starzl et al. 1979t), similar to the late fate of hamster xenografts in rats.

The antibody-resistant liver – Although the liver has long been known to be resistant to humoral rejection (Starzl et al. 1974), it is not impregnable. The majority of hepatic recipients of ABO-incompatible or lymphocytotoxic crossmatch-positive livers survive, but their ultimate prognosis is significantly degraded (Takaya et al. 1992b). Their hepatic allografts have a high incidence of bile duct abnormalities including intrahepatic stricture formation and biliary sludge formation similar to that found in our 2 baboon xenograft recipients (Donaldson et al. 1987, Batts et al. 1988, Demetris et al. 1992) and in hamster→rat xenografts.

Such patients also have an intraoperative liability of fibrinolysis, bleeding, and an excessive consumption of blood products (Weber et al. 1989). This is the liver's

version of a slow-motion hyperacute rejection which may or may not spontaneously abate. The clinical consequences of early hepatic failure and complex clotting disorders have been mimicked in untreated rats (Knechtle et al. 1987b), swine (Merion & Colletti 1990), and Rhesus monkeys (Gubernatis et al. 1987) that were presensitized with donor skin grafts. Although hyperacute rejection did not occur, sensitization markedly reduced the mean survival – in the monkey experiments to 2.5 from 26 days.

As with the kidney, humoral rejection has been documented in livers transplanted into antibody-free recipients. The "slow-motion" nature of the liver's humoral rejection over a period of 2 or 3 days was shown in patients who received contemporaneous kidneys that were hyperacutely rejected within a few minutes (Starzl et al. 1989v). Liver patients who inexplicably reject one liver after the other with no apparent explanation have been seen by us and in other centers, causing the word-of-mouth descriptive term "liver eaters" to be applied. In such cases, a strong association with preexisting or perioperative endotoxemia (which is a classical cause of complement activation) has been noted (Yokoyama et al. 1989).

The baboon→human liver xenografts – Granting that humoral rejection is a complement-activation syndrome of variable expression, the fate of the baboon renal and hepatic xenografts in our human recipients is not mysterious in retrospect. Because the verifying information is unavailable from the 1963 kidney experience, we will confine our remarks here to the 2 recent liver cases.

Postoperatively, total complement in both patients was depleted for most of the critical first 2 weeks while complement components C3, 4, and 5 became undetectable (Starzl et al. 1993d, 1994e). During this time, circulating immune complexes appeared. This complement evolution was similar to that reported previously by Manez et al. (1993) in recipients of allografts transplanted across a positive lymphocytoxic crossmatch. After 10 days, the complement measures returned toward, but never to, normal.

The freedom from cellular rejection in the liver biopsies and at autopsy gave little insight into the complement storm through which both grafts had passed. Sludging as well as the presence of polymorphonuclear leukocytes was seen in the sinusoids of the xenografts immediately after reperfusion, compatible with the diagnosis of an aborted hyperacute rejection (Starzl et al. 1968, 1970, Williams et al. 1968). The hepatocytes exhibited a very fine microsteatosis on the first biopsies, detectable at first only in retrospect, but becoming obvious within a few days, particularly in Case 2. This finding has been reported within 1 or 2 hours postperfusion in cases of *allo*transplantation with inexplicable primary hepatic non-function (Kakizoe et al. 1990). Although the abnormality receded, the microsteatosis was suspected to represent an immunologically mediated sublethal injury that precluded long-term success. The early biopsies also showed widespread

binding of IgM and IgG. The IgM but not the IgG largely disappeared from the graft tissues in later biopsies (Starzl et al. 1993d, 1994e).

Hyperacute xenograft rejection

This is the most extreme example of the complement-activation syndrome. Simonsen began his masterful treatise on allogeneic and xenogeneic graft-versus-host reactions by saying: "*In the whole field of modern transplantation biology, there are few significant contributions which were not made with utter simplicity*" (Simonsen 1962). Xenotransplantation is no exception. Nearly 30 years ago, Perper & Najarian (1966a, b) defined the xenograft barrier by studying the easy sheep⇆goat species combination in which preformed antigraft antibodies were not present (Perper & Najarian 1966b) and the difficult one of pig⇆dog (Perper & Najarian 1966a) in which they were. However, Good and Gewurz and their associates realized that complement activation was the critical issue, whether triggered (classical pathway) or not (alternative pathway) by antigraft antibodies (Clark et al. 1966, Gerwurz et al. 1967). This central principle was even more clearly elucidated by Schilling et al. (1976).

In side-to-side experiments done by us in collaboration with K.A. Porter (St. Mary's Hospital and Medical School, London) and the research team of Frank J. Dixon (Scripps Institute, LaJolla), the hyperacute or slow-motion humoral allograft rejection syndromes seen in clinical practice could be duplicated with allografts in sensitized dogs and with pig⇆dog kidney, liver, and spleen xenografts, varying only in detail (Simpson et al. 1970, Giles et al. 1970, Boehmig 1971). The immunoflourescence and other histopathologic end points with the allograft and xenograft models were indistinguishable, and were preceded by the same kind of coagulopathic changes, binding of antibodies, and sequestration of formed blood elements by these organs.

The valuable encyclopedia of details compiled subsequently about the variable antibodies or target specificities in a variety of xenograft species combinations (Makowka & Cramer 1994, Auchincloss 1988, Plat t et al. 1990, 1991, Weill & Houssin 1994, Bach et al. 1994, Dalmasso 1992) has only added to the original impression that the hyperacute allograft and xenograft rejection mechanisms are fundamentally the same. The intractability of these syndromes to intervention has been rediscovered over and over (reviewed in Makowka & Cramer 1994, Auchincloss 1988, Platt et al. 1990, Platt & Bach 1991, Weill & Houssin 1994, Bach et al. 1994, Dalmasso 1992).

Frustrating recipient treatment strategies

Methods of altering the allograft or xenograft recipient have been recycled since the mid 1960s, usually arousing optimism at first until it was shown that difficult

barriers could not be crossed.

Antibody suppression – Because xenograft rejection is not inherently antibody-dependent, this general approach has a predictably limited place. Splenectomy, which is justified by the spleen's role in antibody responsiveness (Starzl 1964w) prolongs organ survival in several xenograft models, especially when the procedure is combined with T-cell directed immunosuppression (Murase et al. 1993, Valdivia et al. 1987, Monden et al. 1989). The ability to do the same thing pharmacologically was discussed earlier, using drug combinations that suppress humoral as well as cellular immunity. However, in our baboon xenotransplantation cases, the use of splenectomy plus an antibody suppressive drug regimen was ineffective.

Antibody depletion – Plasmapheresis was first shown by Merkel et al. (1971) and Bier et al. (1970) to prolong many-fold the survival of pig kidneys in canine recipients. Such techniques have been used clinically to reduce isoagglutinin titers before allotransplantation across ABO barriers (Alexandre et al. 1991). Technologic improvements such as the staphylococcal protein A adsorbent columns (Bygren et al. 1985) have allowed greater specificity and efficiency of immunoglobulin removal but with ultimately limited value. The temporary transplantation of "forerunner organs" in preparation for the definitive graft has been described with various species combinations with significant but clinically inconsequential protection of kidney, heart, or liver xenografts (Miyazawa et al. 1990, Simpson et al. 1970, Giles et al. 1970, Fischel et al. 1990, Cooper et al. 1988, Tuso et al. 1993). The same discouraging results were obtained in human recipients of kidney allografts (Starzl et al. 1970, Corman et al. 1973) and a chimpanzee hepatic xenografts (Giles et al. 1970). These techniques can not be expected to play a significant role in clinical xenotransplantation.

A reduction of humoral as well as cellular immunity can be accomplished with thoracic fistula in 5 days in rats (Gowans 1959) and >28 days in humans (Starzl et al. 1979u). However, hypersensitized humans conditioned in this way either rejected their kidney allografts hyperacutely or had a slow-motion version which in some cases was accompanied by a systemic coagulopathy and thrombocytopenia that necessitated graft nephrectomy (Weber et al. 1989).

Most recently, complete removal of IgM with monoclonal antibody did not prevent hyperacute heart rejection in the guinea pig→rat model (Soares et al. 1994). This provided evidence of alternative pathway complement activation.

Inhibition of complement cascade – Crude snake venom (Gewurz et al. 1967), the chelating agents sodium citrate (Linn et al. 1970, Kux et al. 1971), and ethylenediamine-tetraacetate (EDTA) (Belitsky et al. 1973) given intraarterially were shown more than 20 years ago to mitigate hyperacute pig→canine kidney rejec-

tion. Interest in this approach lagged until better agents became available that allowed intervention at specific critical levels of the complement cascade: modified cobra venom (Van Den Bogarerde et al. 1991, Leventhal et al. 1993), soluble recombinant complement receptor Type I (Pruitt et al. 1994, Xia et al. 1993), and a sesquiterpene compound called K 76 (Starzl et al. 1994e, Miyagawa et al. 1993). Although all of these products dramatically improve organ survival in difficult xenotransplantation models, continuous infusion is required. Toxicity of the agents or the adverse consequences of complement depletion *per se* limits therapy to a few hours or days. When treatment is stopped, hyperacute rejection promptly supervenes.

Inhibition of the inflammatory response – Used singly or together, platelet-activating factor (PAF) receptor antagonists (Makowka et al. 1987) and prostanoids (Mundy 1980, Makowka et al. 1987) modestly prolong the survival of pig-to-dog and cat-to-rabbit kidney xenografts by slowing the inflammatory/coagulation response to complement activation.

Alteration of the graft

The possibility of making the organ graft a less spectacular immunologic target is an old idea that holds the best hope for clinical application. The first attempts to do this were with occupation of antibody binding sites with recipient F $(ab')_2$ immunoglobulin fragments. Allografts pretreated in this way and transplanted to hypersensitized animals (Shaipanich et al. 1971) and to an antibody-laden human recipient (Corman et al. 1973) resulted in little or no prolongation of survival. The same has been true for pretreated guinea pig hearts transplanted to rats (Gambica et al. 1992).

Two recent strategies designed to alter the cell composition of the graft appear more logical because (as discussed earlier) this is what happens in successfully transplanted allografts and xenografts.

Production of chimeric organs – The feasibility was described earlier of pre-populating baboon xenografts with a human leukocyte population and of the production of full hamster⇆rat chimerism in lethally irradiated rats or hamsters whose GvHD could be controlled with FK 506 (Luis Valdivia, unpublished data). It remains to be seen if incomplete or even full chimerism will change the image of baboon organs enough to make them viewed as allografts by humans.

There is evidence from pilot experiments in our laboratory that this may be a realistic hope (Luis Valdivia, unpublished observation). Under normal circumstances, mouse→rat heterotopic heart and orthotopic liver xenografts are rejected by unaltered recipients in 2 and 7 days, respectively, in much the same way as with the comparable hamster→rat models. However, when mouse donors were

first made fully chimeric with lethal total body irradiation and reconstituted for at least 3 months with rat bone marrow, the livers in 3 pilot experiments were received by rats as if they were allografts, without rejection for more than 2 weeks. The presence of chimerism for shorter periods (3 to 7 weeks) in the hamster→rat models was without effect.

The same experiments with guinea pig⇆rat combinations are in progress. It will be especially important to determine, first, in which combinations cross-species donors can be produced without fatal GvHD, and second, whether this laborious preparation will achieve the desired purpose, particularly with disparate models. If this proves feasible, the preparation of pig organs for human use would be only one step beyond.

Allograft and xenograft repopulation is confined to the hematolymphopoietic lineages, excluding parenchymal and vascular endothelial cells (Starzl et al. 1992l, 1993n, Demetris et al. 1993, Qian et al. 1994). Because the principal receptor sites of complement are found in leukocyte lineages (Roitt et al. 1989), these white cells would not be expected to initiate xenogeneic complement activation. However, endothelial cells also are replete with complement receptors, and whether the failure to replace them will be a fatal flaw in the plan will have to be determined by direct testing. The chance of xenoengraftment of the altered organs should be increased by perioperative infusion of the chimeric bone marrow of the organ donor in the same way as in the current donor leukocyte augmentation trials (Fontes et al. 1994a).

An important organ-specific question concerns the ongoing complement production by baboon hepatocytes of the altered hepatic xenografts. If the species complement restriction demonstrated by Valdivia et al. (1994) is operational (see earlier), this will preclude the use of hepatic xenografts. This possibility also can be clarified by preliminary experimentation in animal-to-animal models.

Humanization of xenografts by gene transfection – The creation of transgenic pig donors whose cells have permanently transfected human complement regulatory proteins is described elsewhere in this volume, and was the most discussed topic at the Second International Xenotransplantation Conference (Cambridge, September 26–29, 1993). Because only one of the factors that contribute to the xenograft barrier is targeted by this approach, the proposed solution seems almost too narrowly specific to be successful. However, with the full human genome in sight, the prospect of creating completely humanized human organs in animals no longer can be dismissed as a visionary illusion.

SUMMARY

Recent discoveries have suggested that the exchange of multiple leukocyte lineages between grafts and host and subsequent long-term chimerism in both is the semi-

nal mechanism of the acceptance of organs transplanted from the same (allografts) or different species (xenografts). This insight suggests new strategies which may allow xenotransplantation, the principal obstacle to which has been humoral rejection. We have defined humoral rejection as a family of complement activation syndromes afflicting allografts and xenografts in which there is a strong (but not invariable) association with preformed antigraft antibodies, invariable evidence of complement activation, histopathologic stigmas of vascular endothelial damage, and a concomitant local or systemic coagulopathy. The generic descriptive term hyperacute rejection is a misnomer because a slow-motion version of the same "humoral" process can occur with some allografts and is the rule with the so-called concordant species xenotransplantations. The pathway of experience and discovery leading to this conclusion shows clearly that the distinction frequently made between allograft versus xenograft humoral rejection does not actually exist in principle, but only in details and intensity.

Breaking down this barrier to xenotransplantation, whether or not it is associated with antibodies, is unrealistic. However, the possibility of avoiding the barrier has been exposed by showing that animal organs can be humanized, with a mixed donor and recipient cell population similar to the chimerism seen in long surviving allografts or even with complete leukocyte replacement. Pilot experiments in rodents suggest that organs from fully xenogeneic chimeras can be made into xenogeneic targets that are no more provocative of complement activation than allografts when they are transplanted into the donor bone marrow species. Although the validity of this concept of organ xenograft preparation is only at the pilot stage of verification, there is reason to suspect that the complement trigger of humoral rejection can be thereby disarmed. If this can be accomplished, independent evidence suggests that cellular rejection can be controlled with conventional T-cell directed immunosuppression, perhaps even with surprising ease. The potential subtle liability of synthetic products of xenogeneic parenchymal cells is not yet known.

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