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The pig as a model in liver xenotransplantation

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Summary — Xenotransplantation is considered to be the best solution to the critical shortage of human donors. Despite its phylogenetic distance, the pig appears to be the most appropriate source of organs for transplantation in humans. Its anatomy and physiology are similar to that of man, it can be raised in specific pathogen free environments and it is available in large quantities. The immunological barrier remains to be overcome, however. Considerable progress in the pathogeny of xenogenic rejection has led to the development of therapeutic strategies and the prospect of clinical xenografting appears realisable in the near future.

heterologous transplantation / natural antibody / complement / hyperacute rejection / endothelial cell

Résumé — Le porc, modèle de xénotransplantation hépatique. La xénotransplantation d’organes apparaît comme une solution logique au problème crucial de la pénurie en organes humains. En dépit de leur éloignement phylogénétique, les porcs semblent constituer la source d’organes la plus appropriée. Leur anatomie et physiologie sont largement similaires à celles de l’homme, ils sont disponibles en quantité illimitée et peuvent être élevés dans des environnements exempts de pathogènes. Cependant la barrière immunologique reste difficile à franchir. Les progrès importants réalisés récemment dans la pathogénie du rejet suraigu ont permis la mise au point de stratégies spécifiques. La xénotransplantation clinique pourrait émerger dans un avenir proche.

xénogreffe / anticorps naturel / complément / rejet hyperaigu / cellule endothéliale

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The use of animal organs for transplantation in humans is considered to be the best solution to the shortage of human donor organs. Liver xenotransplantation offers several advantages (Cooper, 1993). Patients could be referred earlier in the process of their hepatopathy, increasing their chances of a successful transplant. The borderline patients, suffering from advanced hepatic insufficiency, or major hepatic tumors, could be given their chance without jeopardizing the future of other candidates. Finally, xenotransplantation could have a major impact on the treatment of acute hepatic failure, preventing the death of those patients with fulminant hepatitis before a human allograft becomes available.

Clinical xenotransplantation programs were initiated as early as the 1960s, when cadaveric organs were virtually unavailable. Due to their phylogenetic proximity, non-human primates, mostly baboons and chimpanzees, were used as organ donors. However, these trials were stopped because of the poor results obtained with the primitive immunosuppressive protocols, and because of the emergence of clinical allotransplantation. The interest in xenotransplantation research is currently growing again, since the problem of supply versus demand has persisted. Primates are no longer considered to be the best organ donors for humans. Despite its phylogenetic distance, the pig could be an appropriate source of organs. A better understanding of the mechanisms of xenograft rejection, advances in molecular biology, and the possibility of genetically engineering donor animals are likely to provide long-range solutions to the problem in the near future.

PIG TO MAN XENOGRIFT REJECTION: PATHOGENESIS AND THERAPEUTIC IMPLICATIONS

Xenografts may be classified as either concordant or discordant as suggested by Calne (1970). The majority of concordant species (non-human primate to human) are phylogenetically closely related and recipients do not have natural antibodies against the donor antigens. The primary cause of graft loss in these concordant combinations would be cellular rejection, not very different from that seen in allografting. Discordant species (pig to human) are characterized by the presence of xenogeneic natural antibodies (XNA) in the recipient, acting against the donor antigens, which are responsible for hyperacute rejection occurring in the vascularized organs within minutes to hours of transplantation, and which lead to vascular thrombotic occlusion and graft loss.

Natural antibodies in discordant species

Natural antibodies occur without any prior exposure to antigens from other species of animals having taken place. The reason for their presence is not clear, but they are probably directed against bacterial epitopes that cross-react with antigens present on discordant donor endothelial cells. These XNA are specific for glycoprotein targets on porcine endothelium. A triad of glycoproteins (gp), identified as gp 115/135, based on molecular weight, was first described by Platt et al (1990). Additional porcine endothelial cell glycoproteins have been described recently. Their molecular and functional characterization suggests that most of them belong to the integrin family, and one of them has been identified as the von Willebrand factor.

The primary targets for xenoreactive antibodies are oligosaccharides containing the terminal residue Gal (α1-3)Gal. This epitope is highly expressed in non-primate mammals and in New World monkeys, but has not been found in humans, anthropoid apes and Old World monkeys, which synthesize anti-Gal antibodies. This difference in α-galactosyl epitope expression is the result of the differential activity of the
envelope α1-3galactosyltransferase, whose gene was probably inactivated in Old World primates, 20 million years ago, under a selective pressure that might have been exerted by an infectious agent. The immunological barrier against the transplantation of pig organs into humans might be one of the consequences of that evolutionary event (Galili, 1994).

**Overcoming the natural antibody barrier**

Plasmapheresis and more recently immunopheresis using affinity columns prolonged pig-to-baboon renal xenografts. These techniques are hampered by the removal of non-specific immunoglobulins, which could increase recipient sensitivity to infection in the post-transplant period. Donor organ perfusion allows specific binding of the anti-pig XNA on endothelial cells lining the vessels of the perfused organ. We have shown that the perfusion of pig livers is more efficient than the perfusion of pig kidneys at removing XNA (Meyer et al., 1996). An alternative method for the selective removal of XNA is the use of columns carrying specific xenoantigens. Synthetic carbohydrates have also been used in a soluble form in vivo in an attempt to block the binding of the XNA to their endothelial targets (Rieben et al., 1995). Antibody depletion is limited by the rapid return of anti-donor antibodies after treatment. Several strategies may prevent this rebound effect: monoclonal anti-μ chain antibodies and splenectomy. Drugs that suppress B cells such as tacrolimus, rapamycin, deoxyspergualin, brequinar and mycophenolate mofetil have been successfully tested in small animal models.

A potentially exciting way of preventing the binding of XNA to target antigens would be to engineer genetically modified pigs that would not express xenoantigens. α(1,3)galactosyl transferase gene knockout through homologous recombination has been performed in mice, but this technique is not yet available in pigs. Since the removal of the Galα(1,3)Gal epitope from the surface of endothelial cells may generate new antigenic determinants, it could be valuable to convert the pig αGal antigens into human antigens like H substance by transfecting pigs with the human α(1,2)fucosyl transferase gene (Sandrin et al., 1995).

**The role of complement**

Complement plays a pivotal role in hyperacute rejection. Whether it acts via the classical pathway (after antibody binding) or the alternative pathway (independent of the presence of antibody) depends on the species combination in question. The classical pathway of complement activation seems involved in pig to man xenotransplantation. Complement fixation has many effects, the most important being endothelial cell activation.

A few pharmacologic agents may provide an effective systemic inhibition of complement: cobra venom factor, soluble complement receptor type I (sCR1), anti-C5 monoclonal antibodies. The toxicity or side effects of these agents limit however their potential clinical application.

Alternatively, a long term effect can be obtained by expressing regulators of complement activation (RCAs) in genetically engineered pigs. RCAs are species-specific and it has been hypothesized that the expression of human RCAs on pig endothelial cells could prevent hyperacute rejection. Decay-accelerating factor (DAF), membrane cofactor protein (MCP) and CD 59 block the complement cascade at the stages of C3, C3/C5 and membrane attack complex (MAC) assembly, respectively. Expression of human DAF and CD 59 has been achieved in pigs (Cozzi and White, 1995). Preliminary results of xenotransplantation in pig-to-primate models have revealed a
clear protective effect, anticipating important developments in the future.

**Endothelial cell activation**

The natural antibodies of the recipient bind to the endothelial cells of the xenogeneic donor, triggering the complement cascade. The combination of these factors activate the endothelial cells. The procoagulant state of activated endothelium results in part from the loss of anticoagulant molecules such as thrombomodulin and heparan sulfate, and in part from the elaboration of tissue factor (cofactor for the prothrombinase complex) and plasminogen activator inhibitor (which retards thrombolysis). Moreover normal barriers to the efflux of plasma proteins and cells from blood vessels are disrupted. Interstitial hemorrhage, inflammation, vasoconstriction and thrombosis rapidly follow, destroying the graft.

Interfering with endothelial cell activation could be achieved in transgenic pigs by the overexpression of regulators such as thrombomodulin, or by the inhibition of transcription factors involved in the expression of several genes of activation such as NF-kB (Bach et al, 1994).

**Xenograft cellular rejection**

Once humoral mechanisms are overcome, it is likely that cell-mediated rejection will constitute another obstacle to xenotransplantation. An important point is the strength of cellular xenoreactivity as compared to cellular alloreactivity (Auchincloss, 1994). This question was addressed by the mixed lymphocyte reaction using murine systems, and provided evidence for weak xenoreactivity. Whether differences between allogeneic and xenogeneic cell-mediated rejection are only quantitative, or whether there are some new mechanisms in the xenogeneic response remains unclear. This crucial point requires clarification in order to develop efficient immunosuppressive therapies for xenotransplantation.

**BEST CHOICE OF DONORS: NON-HUMAN PRIMATES OR PIGS?**

Evidence indicates that xenotransplantation between concordant species presents fewer obstacles than it does between discordant species. However the use of non-human primates raises today a multitude of issues, relating to the risk of associated zoonoses, the availability and cost of the animals, and ethical issues.

For the above reasons, some researchers are pinning their hope on the pig to fill the gap between supply and demand. The pig is similar in anatomy and physiology to human, it can easily be raised in a specific pathogen-free environment, and it can be used in the production of genetically engineered animals.

**Primates**

Thomas Starzl performed two baboon-to-human liver transplants at the University of Pittsburgh Medical School in June 1992 and January 1993 (Starzl et al, 1993). The rationale for these xenotransplants was for treatment of end-stage liver disease caused by chronic active hepatitis B, which recurs frequently in human grafts, especially when positive viral replication markers are present at the time of transplantation. Baboon livers are thought to be resistant to the hepatitis B virus. The animals were screened to match blood groups with the recipients and an extensive infectious screening was performed.

The first patient was a 35-year-old man who had undergone a post-traumatic splenectomy three years before and who was positive for human immunodeficiency
virus (HIV), but not immunocompromized. The second patient was a 62-year-old man. Both patients underwent orthotopic liver transplants using the piggy-back technique because of the small size of the baboon liver. Immunosuppression involved the use of tacrolimus, steroids, prostaglandin E1 and cyclophosphamide.

The first patient experienced an uneventful post-operative period, the liver function returned to normal during the second post-transplant week. He died on the 70th post-operative day from a subarachnoid hemorrhage resulting from angioinvasive aspergillosis. The second patient, who was more critically ill at the time of transplantation, never regained consciousness and experienced persistent bilirubinemia. He died on day 27 from complications of peritonitis due to a leak from the enteric anastomosis.

Despite this limited survival record, considerable immunological and physiological data were collected as a result of these procedures.

Pigs

An heterotopic auxiliary pig liver transplantation was performed by the Leonard Makowka group at Cedars Sinai Medical Center in Los Angeles, on a 26-year-old patient with fulminant hepatic failure for whom a suitable allograft was not available (Makowka et al, 1995). Circulating XNA were removed prior to transplantation by plasmapheresis and ex-vivo perfusion of donor kidneys. The liver appeared to function for 20 h, but the patient was determined to be brain dead 26 h after transplantation. In the autopsy the graft exhibited hyperacute antibody-mediated rejection, associated with a rapid return of natural anti-pig antibodies, and the activation of the classical and alternative complement pathways.

Ex-vivo liver perfusion has been recently revisited to provide metabolic support for patients with acute hepatic failure, as a bridge toward allotransplantation. Chari et al (1993) reported four cases of ex-vivo pig liver perfusions, one patient being stabilized for ten days before undergoing a successful orthotopic liver allograft. To simplify this procedure, several investigators have tested a bioartificial liver device consisting of pig hepatocytes in a hollow fiber reactor. Some patients treated with plasma exchange and perfusions through the bioreactor survived and received successful liver transplants.

PERSPECTIVES

Currently the principal obstacle to xenotransplants remains humoral rejection. This affects both discordant and concordant xenografts with a variable intensity. A conventional polytherapeutic approach would be theoretically feasible, and new drugs may be effective when included in this type of regimen. This approach would however compromise the recipient immune system to such a point that allografting would remain the first choice of treatment, xenotransplantation being only considered in emergency cases or under special conditions. The creation of transgenic pigs whose cells have permanently transfected human complement regulatory proteins holds promises for the near future, but as only one component of the immunological barrier is modified, this appears too narrowly specific to be completely successful on its own without other forms of aggressive immunointervention. For this reason the induction of specific tolerance to xenografts through mixed chimerism is currently an area of active experimental interest. The imminent prospect of future xenografting raises a number of social and ethical issues. Among them, the appropriateness of xenotransplantation is not questioned, because it is
unlikely that the current organ shortage will improve to the point where the needs of patients awaiting transplants can be fulfilled.

Finally, vigorous debate is under way over the threat that pathogens undetected in donor animals could infect the human recipient, who could spread the infection to other people (Taylor, 1995). Several study committees in the USA and in Europe are drafting guidelines to minimize the danger from animal-derived pathogens. We have to keep in mind, however, that this problem also applies to the field of allografting and has to be considered in the weighing of risk-benefit discussions.

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