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Overcoming the barriers to xenotransplantation: prospects for the future

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Abstract

Cross-species transplantation (xenotransplantation) has immense potential to solve the critical need for organs, tissues and cells for clinical transplantation. The increasing availability of genetically engineered pigs is enabling progress to be made in pig-to-nonhuman primate experimental models. Potent pharmacologic immunosuppressive regimens have largely prevented T-cell rejection and a T-cell-dependent elicited antibody response. However, coagulation dysfunction between the pig and primate is proving to be a major problem, and this can result in life-threatening consumptive coagulopathy. This complication is unlikely to be overcome until pigs expressing a human 'antithrombotic' or 'anticoagulant' gene, such as thrombomodulin, tissue factor pathway inhibitor or CD39, become available. Progress in islet xenotransplantation has been more encouraging, and diabetes has been controlled in nonhuman primates for periods in excess of 6 months, although this has usually been achieved using immunosuppressive protocols that might not be clinically applicable. Further advances are required to overcome the remaining barriers.

Keywords

antibodies; antipig; coagulation; consumptive coagulopathy; genetically engineered; nonhuman primate; pig; xenotransplantation

There is a well-known shortage of organs and tissues from deceased human donors for the purposes of clinical organ and cell transplantation. Although there are over 100,000 patients waiting for a donor organ in the USA today, the number of donor organs that will become available during the current year will be less than 30,000. The discrepancy between

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available transplantable organs and patients on the waiting list grows each year. Despite successful introduction of living related donors for kidney and liver transplantation, marginal (extended criteria) deceased donors and donation after cardiac death, it remains exceedingly unlikely that human organs will fulfill the needs of those who require organ transplantation.

The situation is even worse for patients in need of cell transplantation, such as islet transplantation for those affected by diabetes mellitus. Many of the 2–3 million patients with Type 1 diabetes in the USA would benefit from pancreatic islet transplantation, but clearly the number of deceased human donors available each year (<7000) will not resolve this problem. Indeed, the potential supply of islets from human donors will never be sufficient to treat the millions of patients with diabetes.

What are the alternatives to human organs and cells? Despite recent advances in stem cell biology and tissue engineering, the clinical application of these techniques realistically remains in the distant future. A readily available animal source of organs, tissues and cells for clinical transplantation (cross-species transplantation or 'xenotransplantation') would resolve this problem. There have been a small number of clinical attempts to use animal organs for transplantation during the past century [1] and, in the majority of cases, nonhuman primates were the sources of organs. The results were generally poor, although a baboon liver functioned in a human recipient for 70 days and a chimpanzee kidney supported a good quality of life for almost 9 months. Clinical experience with pigs as the source of organs or cells has been very limited, and the results have been extremely poor.

Although nonhuman primates are phylogenetically closer than other species to humans, for a number of reasons they are not considered to be a suitable source of organs for clinical xenotransplantation [2]. The potentially high risk of crossspecies transmission of infection to humans, difficulties in breeding, organ size disparities and other impracticalities, as well as ethical issues have largely excluded them from further consideration [3]. The pig is now the preferred source animal and the advantages and disadvantages of this animal have been discussed previously [2-4]. The advantages are many, but the primate immune response to transplanted pig organs and cells has proven to be a significant barrier that as yet may not have been fully overcome [5].

In this review, we shall briefly summarize the immunobiology of xenotransplantation based on current data, and discuss the major remaining obstacles. We shall concentrate our attention on the most relevant experimental model, namely pig-to-nonhuman primate xenotransplantation.

Immunobiology of pig-to-nonhuman primate transplantation

Hyperacute rejection

In initial experiments, when wild-type pig organs were transplanted into nonhuman primates, the binding of natural (preformed) antibodies to the pig vascular endothelium initiated activation of the complement cascade (Figure 1) [6-10]. The endothelial cells of the graft responded to the immune activation by converting from an anticoagulant to a procoagulant phenotype [11,12]. The result of activation of the complement and coagulation systems was hyperacute rejection.

Acute humoral xenograft rejection

When steps were taken to prevent hyperacute rejection (e.g., the depletion of antipig antibodies or complement from the nonhuman primate serum [7,13-22]) a delayed form of antibody-mediated rejection occurred, known variously as acute humoral xenograft rejection

(AHXR), acute vascular rejection or delayed xenograft rejection (Figure 1) [23]. Natural antibody binding and complement activation resulted in vascular endothelial cell activation and injury caused by the complement and cellular components of the innate immune system. There is increasing evidence that primate neutrophils may be involved in pig endothelial cell activation [24-26], a topic that has been discussed previously by our group [27]. Natural killer (NK) cells play a role in AHXR [28-30], as do macrophages [31], but their exact importance remains unclear. AHXR may occur despite the administration of pharmacologic immunosuppressive agents, and is particularly seen following the development of a T-cell-dependent elicited antibody response.

Acute cellular rejection

Potent pharmacologic agents can largely prevent acute cellular rejection (i.e., T- and B-cell infiltration of the graft and T-cell activation) and a T-cell-dependent elicited antibody response, even though the T-cell response is believed to be stronger than the alloresponse [32-36]. This is possibly because T-cell activation leads to a rapid antibody response that results in AHXR before significant T-cell infiltration occurs in the graft. Acute cellular rejection is therefore typically not seen with intense immunosuppressive drug regimens [37-42]. Costimulation blockade agents, such as an antihuman CD154 monoclonal antibody, have been found to be particularly effective in preventing T-cell activation in the xenotransplantation setting [43].

Chronic rejection

In grafts that survive for more than a few weeks, features of chronic vasculopathy develop, similar to the chronic rejection seen in long-surviving allografts (Figure 1). The causative factors remain poorly understood.

Genetically engineered pigs

Most of the advances that have been made in this field have resulted from the introduction of genetically engineered pigs. The most significant advances to date have been the production of pigs expressing a human complement-regulatory protein (e.g., human decay-accelerating factor [CD55], membrane cofactor protein [CD46] or CD59 [44-48]) and pigs in which the gene for α1,3-galactosyltransferase has been knocked out (α1,3-galactosyltransferase gene-knockout [GTKO] pigs) [49-52].

The presence of a complement-regulatory protein on the surface of vascular endothelial cells in the pig largely protects the graft from hyperacute rejection. Of interest is the suggestion that overexpression of a pig complement-regulatory protein might be as effective as the expression of a human complement-regulatory protein in protecting the pig cell from lysis [53], although this theory has never been tested in an *in vivo* model.

The gene for α1,3-galactosyltransferase enables this enzyme to add Galα1,3Gal (Gal) oligosaccharides to various underlying glycoproteins and glycolipids in the pig [54,55]. Gal is the major target for human and nonhuman primate antipig antibodies [49,56,57] (reviewed in [58]), and its deletion from pigs has greatly reduced the incidence of hyperacute rejection of pig grafts in nonhuman primates [59-61]. GTKO pigs additionally transgenic for a human complement-regulatory protein provide increased benefit over each modification alone [62]. IgM, IgG and complement deposition in grafts is absent or less marked, and innate cellular infiltration has also been minimized.

Genetically engineered pig hearts placed heterotopically in baboons have functioned for periods of 3–6 months [59,60,63-65], life-supporting kidneys for periods approaching 3 months [38,39,61,66], livers for a matter of days [67,68] (reviewed in [69]), and lungs for a

matter of hours [70,71]. Progress during the past 20 years or so can be illustrated by the prolongation of survival of pig hearts transplanted heterotopically into baboons using wild-type or genetically modified pigs and various immunosuppressive protocols (Figure 2) [72].

Although the availability of GTKO pigs has been a major step forward, there are well-documented natural antibodies to nonGal antigens in humans and nonhuman primates [29,73-77], the nature of which remains unknown. Although there were reasons to believe that these may be directed to non-Gal oligosaccharides [78,79], recent data from our own center suggest that, with one exception, these antibodies are not directed to carbohydrate structures [Yeh P*et al*., Manuscript In Preparation]. The exception are antibodies directed against *N*-glycolylneuraminic acid (NeuGc).

It has been known for some time that antibodies in humans directed to NeuGc may play a role in pig-graft destruction [80-84]. NeuGc is expressed on the vascular endothelium of all mammals with the exception of humans; chimpanzees also express this oligosaccharide. Although it may play a role when pig organs or cells are exposed to human serum, it cannot be a factor in the destruction of pig organs or cells after exposure to nonhuman primate serum. In pig-to-nonhuman primate transplantation models, therefore, other antigens must be the targets for anti-nonGal antibodies. These anti-non-Gal antibodies, whether directed to NeuGc or other antigens, are weaker and less destructive than anti-Gal antibodies, but nevertheless can be associated with hyperacute rejection or AHXR [41,85].

Genetic modifications to inhibit NK cell [85-88] and macrophage activity [89-91] are possible, but have not yet been tested in a pig-to-nonhuman primate model. Transgenic expression of HLA-E or HLA-G on porcine endothelial cells is known to inhibit NK cell cytotoxicity and adhesion [92,93], and HLA-E transgenic pigs have recently been produced [94]. The intraspecies incompatibility of the inhibitory interaction between CD47 and SIRP-α contributes to the phagocytosis of pig cells by primate macrophages [95,96].

Despite the absence of hyperacute rejection and classic AHXR, survival of pigorgan grafts in nonhuman primates is currently limited by either the development of a thrombotic microangiopathy [40,97-99] or a consumptive coagulopathy, or both [100-103]. These are clearly features of coagulation dysregulation between pig and primate, and this barrier has not yet been overcome. Following GTKO pig heart transplantation in baboons, thrombotic microangiopathy is the predominant feature, with subsequent consumptive coagulopathy in some cases [59,60]. However, after GTKO/CD46 pig kidney xenotransplantation, consumptive coagulopathy occurs relatively early in the absence of obvious features of thrombotic microangiopathy [Lin CC, Ekser B, Long C*et al*., Manuscript In Preparation]. Following GTKO/CD46 pig liver xenotransplantation, thrombocytopenia develops within minutes and, although most coagulation parameters appear to remain within the normal ranges, the lack of platelets leads to spontaneous internal hemorrhage within days [68]. Pig lung xenotransplantation or *ex vivo* lung perfusion with human blood provides an accelerated sequence of events, as coagulation dysregulation occurs within minutes or hours [70,71].

Increasing experimental evidence suggests that the classic immune response is no longer the major problem, but physiologic incompatibilities between the coagulation systems of pig and primate are more problematic [102-112]. However, the immune response, particularly that of the innate immune system [27], may be playing a role in initiating this process.

Coagulation dysregulation

Despite considerable attention in recent years, the exact mechanisms by which coagulation disorders develop after xenotransplantation remain elusive. Previous reports suggested that

consumptive coagulopathy is initiated by the expression of tissue factor (TF) in the porcine graft [113,114]. In response to the binding of xenoreactive antibody and/or activation by complement, endothelial cells in the graft are activated to increase TF activity and initiate intragraft thrombosis and consumptive coagulopathy [11,23].

During inflammation, type I activation of endothelial cells induces P-selectin and vascular leakiness of plasma proteins; this process takes 10–20 min. Type II activation of endothelial cells is triggered by the stimulation of TNF-α and IL-1, induces more effective leukocyte recruitment by synthesis of adhesion proteins, such as E-selectin and CD106 (VCAM-1), and is sustained for 6–24 h after cytokine-mediated activation. Type I and type II activations are believed to be associated with hyperacute rejection and AHXR, respectively [11]. The activated endothelial cells and the generated thrombin subsequently activate platelets, leukocytes and other inflammatory cells in the recipient, initiating a vicious cycle.

Recent *in vitro* studies at our center by Lin *et al.* have indicated that porcine aortic endothelial cells (PAECs) are able to induce human TF exposure on human platelets and monocytes through an immune response-independent pathway [115]. We have investigated this problem *in vivo* in pig-to-baboon kidney [Lin CC, Ezzelarab M, Shapiro R*et al*., Manuscript In Preparation] and liver [Lin CC, Ekser B, Long C*et al*., Manuscript In Preparation] transplantation models. For example, the rapid development of consumptive coagulopathy in a pig-to-baboon liver xenotransplantation model has been studied [Lin CC, Ekser B, Long C*et al*., Manuscript In Preparation]. Using genetically modified pig liver transplantation into baboons, we observed that there is a massive loss of platelets from the circulation within minutes after reperfusion [68]. The development of trombocytopenia was accompanied by thrombin formation. Circulating platelets and peripheral blood mononuclear cells expressed functional TF and aggregated in the graft without the documented activation of donor endothelial cells (confirmed by negativity for P- and E-selectin, CD106 and TF expression on the porcine endothelial cells by immunofluorescence staining) [Lin CC, Ekser B, Long C*et al*., Manuscript In Preparation]. Although there was a minimal measurable immune response (indicated by a lack of antibody and complement activity), consumptive coagulopathy still occurred. The severity and rapidity of thrombocytopenia were not alleviated by manipulation of the immune response (e.g., by prior depletion of complement by the administration of cobra venom factor). We therefore tentatively concluded that recipient TF initiated consumptive coagulopathy by a mechanism that is independent of the immune response.

These observations suggest that further manipulation of the immune response (with the increased risks of infection and other complications) will not completely overcome consumptive coagulopathy after xenotransplantation. Determination of the exact mechanism by which thrombotic microangiopathy and consumptive coagulopathy are initiated after xenotransplantation is important because it may enable further genetic modification in the pig or suggest therapy that might prevent them. The introduction of genes for human thrombomodulin, TF pathway inhibitor [116] or CD39 [117] have been suggested to overcome the coagulation incompatibilities between pig and primate.

Pig pancreatic islet xenotransplantation

In the field of porcine islet transplantation in diabetic nonhuman primates, the challenges are slightly different, and greater progress has been made.

Adult porcine islets do not express Gal [118,119], thus reducing the antibody-mediated response to them after transplantation into a primate. Fetal and neonatal islets do express Gal [120], and so GTKO pigs are likely to be advantageous. However, within purified adult pig islets remain fragments of vascular endothelial cells that express Gal, and it is possible that

the immune response to these may be detrimental to survival of the surrounding islets. Therefore, it would seem advantageous to use GTKO pigs as the sources of all islets (fetal, neonatal and adult). Even though anti-Gal antibodies may not play a major role in islet graft rejection, it has been demonstrated that antibodies directed to unknown non-Gal antigens may be important [121,122].

When the islets are transplanted into the portal vein so that they reside within the liver (the current approach in clinical islet allotransplantation), there is a major loss of islets from what is known as the 'immediate blood-mediated inflammatory reaction' (IBMIR) (reviewed in [123]). Although IBMIR occurs following islet allotransplantation, it would appear to be of greater magnitude after pig islet xenotransplantation. It appears to be a nonspecific response to the presence of islets in the blood where, of course, they are normally not present. It involves both complement activation and activation of the coagulation system, and rapidly leads to destruction of a large number of islets either through complement activity or through ischemia following thrombus formation around the islets. The loss of islets is estimated to be in the region of 60–80%. However, there is also evidence that antibody-mediated complement activation may be playing a role [124].

If enough islets survive this attack – possibly a relatively small number – then normoglycemia may result. Pig islets that express the human complement-regulatory protein, CD46, appear to provide some protection from this response or from the antibody-mediated complement activation that occurs, but it is not yet certain how important CD46 expression is in contributing to the prolonged survival of CD46 pig islets reported in monkeys [125].

Even if sufficient islets remain viable after IBMIR to maintain a state of normoglycemia for a period of time, if the number of islets surviving is borderline, then islet function may fail and hyperglycemia will gradually return. It is currently unclear whether this slow loss of control of glycemia is related to immune system activity or just to physiologic 'exhaustion' of the islets.

T cells would appear to play a greater role in the rejection of pig islets than of pig organs. It would therefore appear to be even more important to suppress the T-cell response after pig islet xenotransplantation than after pig organ xenotransplantation. Fortunately, there are regimens that can do this. For example, at our own center, we have had encouraging results using a regimen consisting of induction therapy with antithymocyte globulin, and maintenance with an anti-CD154 monoclonal antibody and mycophenolate mofetil [125,126]. Others have had equally good results, but with more intensive immunosuppressive regimens [127,128].

An attempt is currently being made by several centers to reduce the intensity of the immunosuppressive therapy required and, in particular, to use agents that are clinically available at the present time or certainly will be in the near future. For example, at our own center, we are trying to replace anti-CD154 monoclonal antibody with another costimulation blockade agent, CTLA4-Ig. A regimen of antithymocyte globulin, CTLA4-Ig and mycophenolate mofetil would be clinically acceptable.

Immunological tolerance

The induction of immunological tolerance to the graft is the ultimate and ideal goal for xenotransplantation (and allotransplantation). Considerable efforts have been made to achieve this goal either by pig bone marrow transplantation (to induce mixed chimerism) (reviewed in [129]) or by pig thymus transplantation in the host [61,130]. After kidney allotransplantation, the induction of mixed chimerism, even if only transient, has been

associated with the induction of tolerance to the graft in both nonhuman primate [131] and clinical models [132]. To date, however, neither of these approaches has been convincingly successful in models of xenotransplantation.

There is increasing interest in the potential role of T-regulatory cells (reviewed in [133]) and/or mesenchymal stem cells to induce a state of tolerance to a xenograft, but to date there has been very little exploratory work reported. The possibility of inducing B-cell tolerance in neonates, as has been achieved in ABO blood group-incompatible allografts [134,135], is also intriguing [136]. However, there are obviously a number of other barriers, such as thrombotic microangiopathy and consumptive coagulopathy, which need to be overcome before tolerance is likely to be induced.

In this review, we have not considered other areas of importance to clinical xenotransplantation. These include, first, the physiology of pig organ and cell grafts in primates and, second, the potential risk of infection with a pig microorganism that might be transferred to the recipient. These two topics will be very briefly discussed.

Pig organ function in primates

Even if the immunologic and coagulation barriers can be overcome, the question has been asked as to whether a pig organ will function satisfactorily in the primate bodily environment. Will the organ carry out all of the functions required of it (i.e., all of the functions of a native primate organ?). The physiological aspects of xenotransplantation have been reviewed relatively recently [137].

In summary, current evidence is that pig hearts function well in primates. Successful orthotopic life-supporting pig heart transplantation in baboons has been followed by satisfactory function for periods of up to 53 days [138,139]. The pig heart has been demonstrated to recover from an initial ischemic injury occurring during the transplant operative procedure [139].

Pig kidneys function adequately with one or two possible exceptions (e.g., handling of phosphate [140]). However, one major problem following pig kidney transplantation in nonhuman primates is the development of proteinuria, which can be considerable. This results in albuminemia with its accompanying complications, such as peripheral edema. Although this can be prevented or corrected by the continuous intravenous infusion of human albumin, this would clearly not be a realistic long-term therapeutic option in a patient with a pig kidney graft. Whether the proteinuria is related to the immune response or is simply a physiologic incompatibility remains uncertain. Our own observation that it develops rapidly (within hours) in the absence of significant antibody or complement deposition in the graft suggests that it may not be immune related.

Evidence for satisfactory function of the pig liver in a primate is limited and inconclusive. In addition to its detoxification functions, the liver synthesizes approximately 2000 different proteins, and it is unlikely that all the products of a pig liver will function adequately in a primate. However, we have evidence from our own studies that detoxification by a pig liver after orthotopic transplantation into a baboon is adequate, proteins are synthesized and that pig coagulation factors are produced that appear to function adequately in the primate [68]. If it is determined that one or two key or essential pig proteins do not function in the primate host, then it may be possible to genetically engineer the pig to produce the desired human protein.

The level of serum albumin in pigs is significantly lower than in primates. After pig liver transplantation, we observed that the albumin level falls from that seen in the baboon to that

seen in the pig. The level can be maintained by the intravenous infusion of human albumin, but this again would be problematic if long-term albuminemia persisted.

Potential risk of transfer of a porcine microorganism to the human recipient (xenozoonosis)

The potential for the development of a xenozoonosis in the recipient of a pig graft (i.e., the potential for a porcine microorganism to cause infection in the recipient) has been of concern for a number of years [141-143]. These potential risks, particularly with regard to porcine endogenous retroviruses (PERV), are now considered to be much less significant than they were a few years ago [142-145], and a clinical trial would be deemed justified if there were a realistic possibility that the graft would be life-saving for the patient. Furthermore, activation of PERV can now be prevented by siRNA technology [146,147], although this is unlikely to be necessary. Nevertheless, largely because of the possibility of the transfer of a porcine-infectious microorganism, xenotransplantation will be highly regulated by national regulatory authorities, such as the US FDA. The likely regulatory requirements have recently been reviewed by Schuurman [148].

Clinical perspective

There are clearly problems that remain to be overcome before pig organs can be used in clinical transplantation, although pig islet transplantation is much closer to being translated into the clinic. As truly long-term survival of pig organ grafts may be limited for some time by the early onset of graft atherosclerosis or other forms of chronic rejection (until this problem can be resolved), initial clinical trials may involve ‘bridging’ a patient in end-stage organ failure, particularly of the liver [149] or heart [150], until a suitable allograft becomes available. This would not only be lifesaving – and therefore ethically justified – but would also enable valuable experience of pig organ function in humans, as opposed to nonhuman primates, to be gained.

However, ‘bridging’ would not be a clinical option if sensitization to pig antigens (e.g., swine leukocyte antigens), resulted in an increase in panel-reactive antibodies (i.e., antibodies to HLA), which might either preclude subsequent allotransplantation or be detrimental to the outcome of such a procedure. Fortunately, although limited, current evidence is that antibodies that develop after exposure to a pig xenograft (if immunosuppressive therapy has been unsuccessful in preventing sensitization) are not cross-reactive against HLA, and so would not be detrimental to a subsequent allograft (reviewed in [151]). By contrast, patients with a high level of HLA-reactive antibodies may be at greater risk of rejecting a pig xenograft, although again the evidence for this remains limited (reviewed in [151]).

The potential therapeutic possibilities offered by xenotransplantation are so considerable that it remains an area of research that should be pursued vigorously until the barriers have been overcome. Not only will pig organs and islets offer therapeutic options, but there are potential therapies related to pig corneal transplants, pig neural-cell transplants (in conditions such as Parkinson’s and Huntington’s disease), and even pig red blood cells for transfusion into humans [152]. The number of patients who might benefit from xenotransplantation may therefore run into the hundreds of thousands or even millions if it can achieve its potential.

Expert commentary & five-year view

The increasing availability of genetically modified pigs is steadily drawing clinical xenotransplantation closer. Treated 'nonviable' tissues from wild-type pigs, such as dermis scaffolds and small intestinal stroma, are already being used on a large scale in clinical surgery, and steps are underway to improve outcomes by using GTKO pigs for these purposes. There is evidence to indicate that tissues from GTKO pigs will generate a weaker inflammatory response in the recipient.

Work at our own and other centers is exploring the potential of pig corneas for corneal transplantation, and we have also investigated the possibility of using GTKO pig red blood cells for clinical transfusion [152]. The encouraging results of pig islet transplantation in diabetic monkeys [127,128], particularly when islets from genetically engineered pigs are transplanted [125], suggest that clinical islet xenotransplantation is almost certain to be instituted within a few years. Pig organ transplantation in patients with end-stage organ failure is likely to follow, initially as a bridge to allotransplantation.

In summary, therefore, further genetic engineering of pigs is required to protect the organs and islets from the primate immune response, particularly from the innate immune system. Most importantly, genetically engineered pigs are required whose organs and cells are protected from the coagulation dysregulation that occurs. In particular, modifications are required to prevent, first, TF activity on the graft [Lin CC, Ezzelarab M, Shapiro R*et al*., Manuscript In Preparation] and, second, activation of recipient platelets to express TF and initiate consumptive coagulopathy [112,115].

An immunosuppressive regimen is required to prevent cellular rejection and a T-cell-dependent elicited antibody response, and this regimen must be one that is clinically applicable and not associated with a high incidence of complications, such as infection or malignant disease. In this respect, an alternative is to express the immunosuppressive agent in the graft. For example, CTLA4-Ig has been very successfully expressed ubiquitously in pigs – so successfully, in fact, that this resulted in complications of immunosuppression in the pig [153].

In 5 years time, therefore, we anticipate that clinical trials of islet xenotransplantation will have been initiated. The availability of GTKO/CD46 pigs transgenic for a human antithrombotic or anticoagulant gene will have resulted in improved organ graft survival in nonhuman primates, and may allow consideration of clinical trials of bridging to allotransplantation.

Key issues

- Genetic engineering of pigs to prevent the coagulation dysfunction that occurs between a pig organ graft and recipient primate may be achieved by the expression of thrombomodulin, tissue factor pathway inhibitor, CD39 or other mechanisms in the pig vascular endothelium.
- Determination of an effective immunosuppressive regimen that is not so intensive that it results in complications, such as infection or malignancy, can be achieved by T-cell costimulation blockade, which offers great potential towards this goal.
- Protection of pig islets from the instant blood-mediated inflammatory reaction following transplantation into the portal vein may be achieved by expression of anticomplement and anticoagulant genes on the islets. Alternatively, a different

site for islet transplantation, such as the gastric submucosal space, should be explored.

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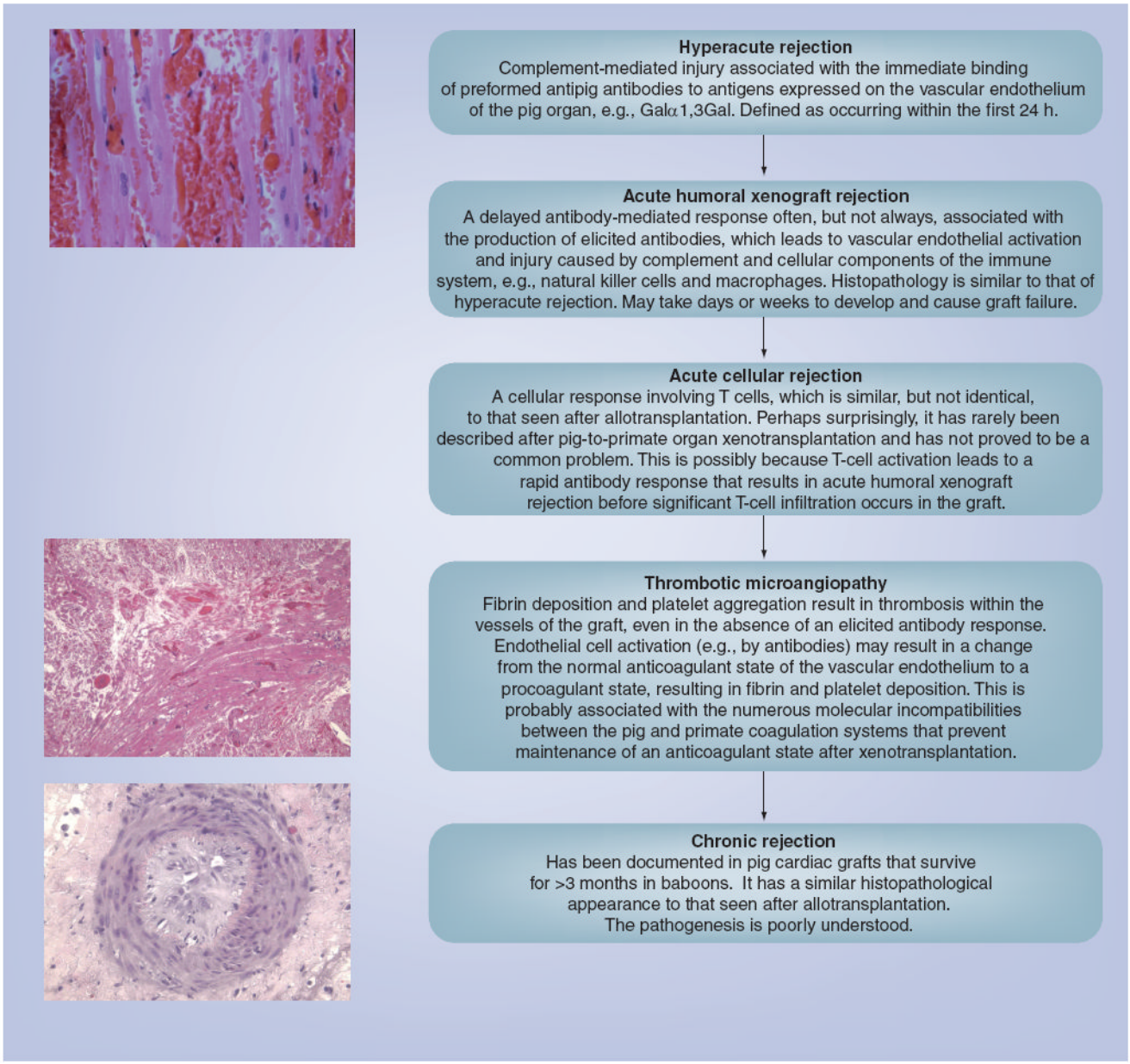

Figure 1. Summary of the major known immunologic barriers to pig-to-primate organ transplantation, as exemplified in the transplanted pig heart

In hyperacute rejection, the graft develops microvascular thrombi, beginning in the venules. Occlusion of the vessels leads to rupture with interstitial hemorrhage and edema. Some cells of the innate immune system, such as neutrophils and macrophages, may be present. The appearance in acute humoral xenograft rejection is similar, although infiltration by cells of the innate immune system is more pronounced. Isolated acute cellular rejection is very rare, but T cells can be seen when thrombotic microangiopathy develops. The role of T cells in the development of thrombotic microangiopathy is uncertain and controversial. Reproduced with permission from [72].

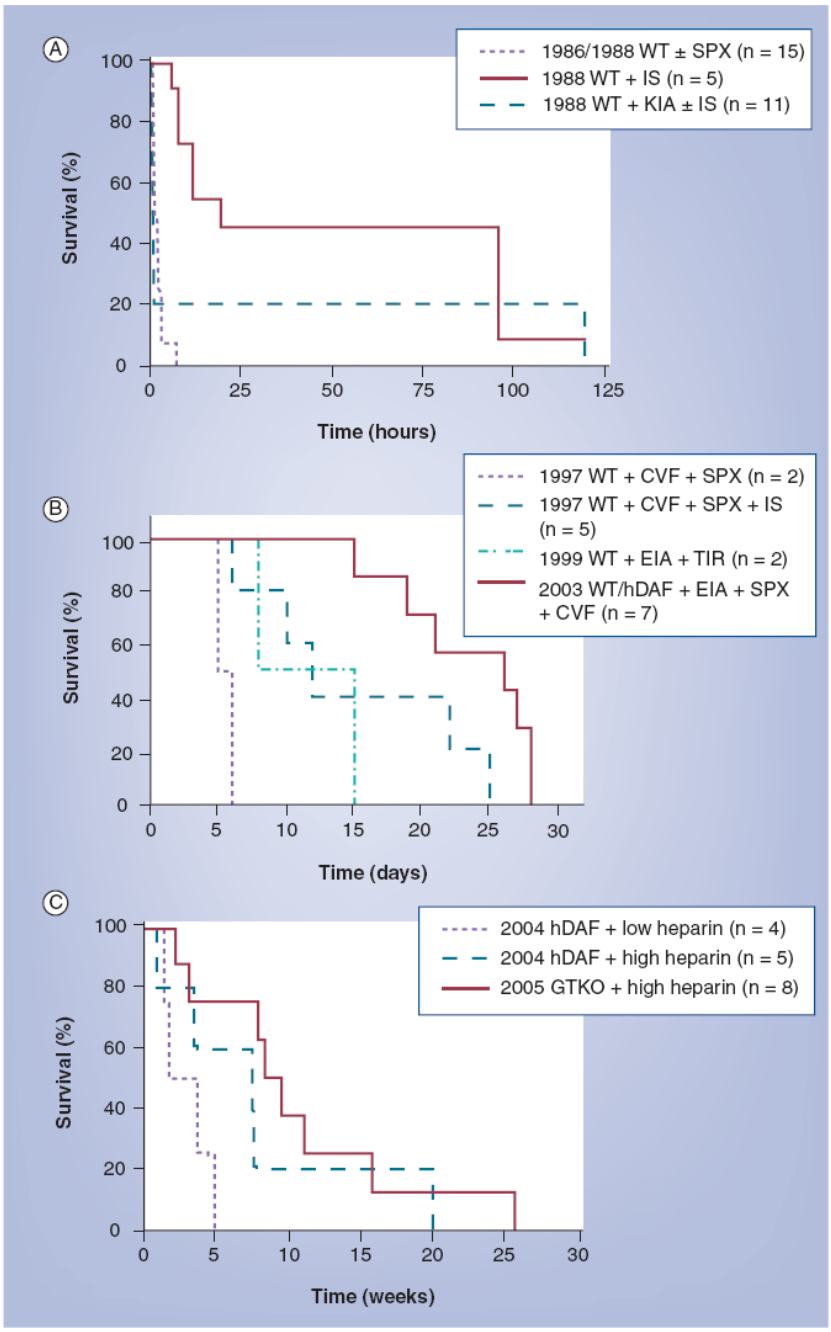

Figure 2. Progress in the results of pig heterotopic heart transplantation in baboons (1986–2005)

(A) Survival (in hours) of selected pig heterotopic heart grafts in baboons (1986–1996). **(B)** Survival (in days) of selected pig heterotopic heart grafts in baboons (1997–1999). **(C)** Survival (in weeks) of selected pig heterotopic heart grafts in baboons (2000–2005).
CVF: Cobra venom factor; EIA: Extracorporeal immunoadsorption; GTKO: α1,3-galactosyltransferase gene-knockout; hDAF: Pig transgenic for human decay-accelerating factor; IS: Pharmacologic immunosuppressive therapy; KIA: Prior pig kidney perfusion to deplete antipig antibodies; SPX: Splenectomy; TIR: Tolerance-inducing regimen; WT: Wild-type.
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