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Organ shortages have led to long waiting lists for organ transplantation. One alternative to allotransplantation is the use of animal organs, that is, xenotransplantation. Transplanting across species invokes a powerful set of immune responses and may expose donors to previously unseen infectious agents. Whereas the problem of hyperacute rejection has received much attention, less time has been devoted to the cellular immune response to xenografts. This obstacle, as well as the risk of infectious disease transmission, has become an important focus of research and is reviewed here.

Numerous patients have been exposed to porcine tissues for therapeutic indications such as pancreatic islet xenografting and extracorporeal liver or kidney perfusions.

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Introduction

In the United States each year, many people die awaiting an organ transplant. In 1999, there were 72,110 registrants on the Organ Procurement and Transplantation Network waiting list. The median wait for a kidney transplant in 1996 was 1033 days for men and 1196 days for women. The death rate was 78.5 per 1000 patient years at risk in 1999 for those on the kidney transplant list.[1] These startling statistics highlight the need for increased public awareness and support for organ donation. A second approach to addressing this shortage is the use of xenotransplants, grafts from other species.

For anatomical, practical, and ethical reasons, the pig has emerged as the animal of choice for human xenotransplantation. Although there have been a handful of clinical attempts, a formal program of pig-to-human transplantation is still years away. Critical to the development and implementation of such a program is a detailed understanding of the immune mechanisms responsible for xenograft rejection (Fig. 1). As laboratories investigate immunity in allotransplantation, a parallel effort exists for xenotransplantation.

Humoral Immunity

Hyperacute rejection occurs immediately upon transplantation of pig organs into a primate host. This is due to the presence of preformed xenoreactive natural antibodies (XNAs). Pig cells express several epitopes, most importantly Galα1-3Gal, that are not found in old-world primates. These primates, including humans, do not express the gene encoding this residue and develop antibodies to it shortly after birth.[2] The antibodies are most likely formed in response to bacterial antigens encountered as normal environmental flora.[3] Upon transplantation, preformed antibodies in the recipient bind avidly to these antigens on endothelial cells. The complement system is activated, with resultant interstitial hemorrhage and formation of platelet thrombi,[4] leading to rapid organ loss. In addition to causing hyperacute rejection, XNAs appear to activate porcine endothelium, potentially priming it for later cellular rejection.[5]

Several strategies have been proposed to prevent hyperacute rejection. Depletion of recipient xenoreactive antibody was shown to prolong xenograft survival.[4] Similarly, transgenic pigs deficient in expression of xenoantigens[6] or presenting modified antigens[2,7] are protected from xenoantigen-mediated hyperacute rejection. Inhibition of complement has been attempted through infusion of inhibitors[2,8] or by transgenically modifying porcine cells to express human decay accelerating factor[9] or CD59.[10]

Partially due to the work mentioned above, the problem of hyperacute rejection is much closer to being solved. However, this leaves the transplanted organ vulnerable to cellular rejection. Much less is known about the detailed mechanisms of cellular rejection. It is clear that many elements of the immune system cooperate in the rejection of xenografts, and a greater understanding of their interactions is needed. If we are able to overcome the hurdles of cellular rejection and maintain a viable

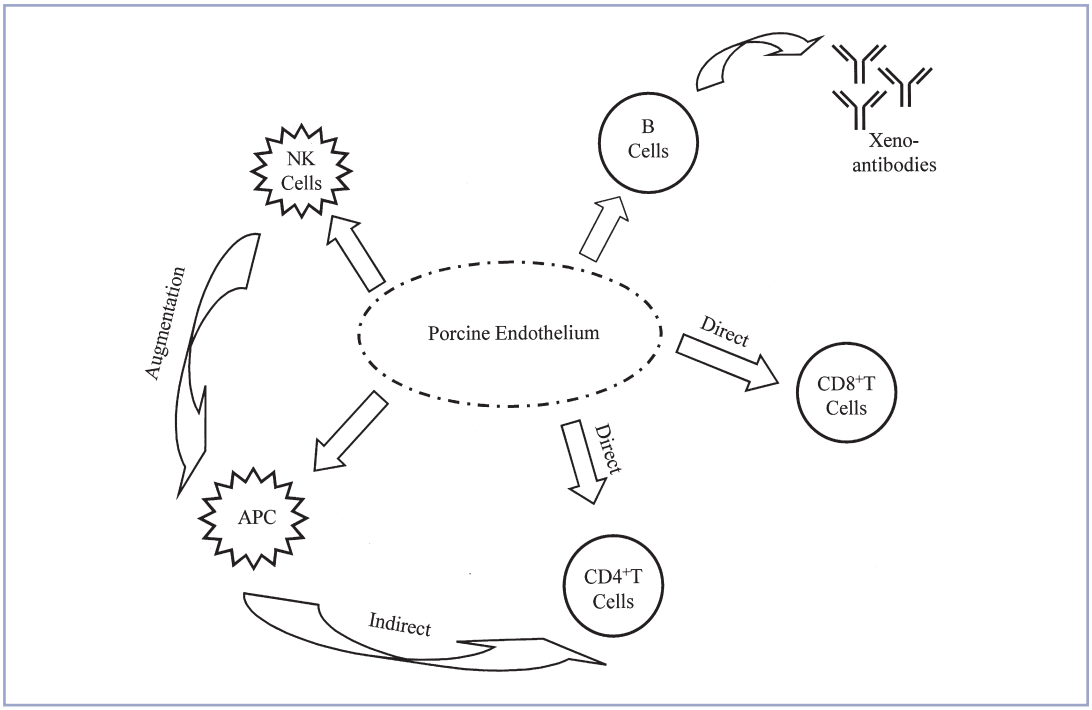

Figure 1. Humoral and cellular immune interactions between human cells and porcine targets (APC = antigen-presenting cell, NK = natural killer).

xenograft, concern will shift to the possibility of infectious disease transmitted to the recipient.

Cellular Immunity

Our laboratory has focused on elucidating the human cellular immune response to porcine xenografts. Beyond the period of hyperacute rejection, $CD4^+$ and $CD8^+$ T lymphocytes contribute to xenograft rejection. Normally, $CD4^+$ cells recognize foreign peptides presented by self HLA class II, and $CD8^+$ cells recognize HLA class I molecules with their associated peptides. Thus, after xenotransplantation, 2 pathways of antigen recognition are possible: direct and indirect. In the direct pathway, host $CD4^+$ and $CD8^+$ T lymphocytes recognize donor major histocompatibility complex (MHC) class II and I antigens directly on parenchymal cells of the xenograft. In the indirect pathway of immune recognition, host antigen-presenting cells (APCs) take up xenoantigen and, after processing, present it to $CD4^+$ T lymphocytes in the context of self HLA. Both of these pathways appear active in xenotransplant models.

In the xenoreactive immune response, the indirect pathway of antigen recognition has traditionally been considered the more important of the 2 pathways. The proliferative response of $CD4^+$ cells to porcine aortic endothelial cells (PAECs) was significantly increased in the presence of human monocytes (APCs), and this response was blocked by the addition of antibodies directed against HLA class II. Treatment with chloroquine caused a marked inhibition of T-cell proliferation, indicating that processing of xenoantigens is necessary for $CD4^+$ T-cell activation.[11] The addition of IL-10 also abolishes the proliferative response, possibly by inhibition of the antigen-presenting function of monocytes.[12] Indirect recognition has also been demonstrated against porcine islet cells. T-cell lines generated against porcine islets were primarily $CD4^+$ and recognized peptides derived from class I swine leukocyte antigens presented by self APCs.[13]

Recent studies have attempted to determine the epitopes involved in indirect recognition. Across haplotypes, the swine leukocyte antigen (SLA) molecule differs primarily at the hypervariable re-

gion. Peptide epitopes were synthesized from this polymorphic area and pulsed to APCs. $CD4^+$ T lymphocytes proliferated in response to both whole SLA class I molecules and the peptide epitopes derived from the polymorphic SLA region when incubated with APCs. This suggests that the SLA molecule itself, and specifically the hypervariable region, can be an immunogenic xenoantigen (unpublished data).

In the direct pathway, $CD4^+$ and $CD8^+$ lymphocytes recognize SLA molecules and are able to interact with them similarly to HLA.[14] Our studies have shown that human $CD8^+$ T cells directly recognize PAECs in an SLA class I restricted fashion.[15] Stripping PAECs of SLA-bound peptides significantly reduced target-specific lysis by T cells, and addition of haplotype-specific peptides restored lysis. Further, transfection of a relevant SLA class I allele into an HLA class I negative cell line resulted in $CD8^+$ T-cell-mediated lysis when the appropriate porcine peptide was added.[16] $CD4^+$ T cells have also been shown to proliferate in direct response to SLA class II molecules and appear to depend on the CD28 costimulatory pathway, with porcine B7 as the ligand. Incubation with CTLA-4-Ig (which binds porcine B7) inhibited proliferation.[16] In this regard, transfection of porcine CD86 (B7) into Chinese hamster ovary cells and human umbilical vein endothelial cells induced proliferation and cytokine production by human xenoreactive $CD4^+$ T cells.[17] PAECs were also shown to ligate CD2 on human $CD4^+$ T cells, since blockade of this interaction inhibited T-cell proliferation.[18]

Several reports have indicated that human natural killer (NK) cells can infiltrate species-discordant transplanted organs and may therefore play an important role in xenograft rejection.[19,20] HLA class I molecules provide inhibitory signals to NK cells, sparing them from NK-cell-mediated lysis. HLA-C is a classical MHC molecule that protects cells from lysis by ligation of a killer cell inhibitory receptor (KIR). In addition to the recognition of classical MHC molecules, HLA-E and HLA-G are nonclassical HLA antigens that activate the inhibitory pathway. There is some evidence that SLA class I expression by donor cells can also protect them from NK-cell-mediated lysis.[21] Transgenic expression of HLA-Cw3, but not HLA-A2 or HLA-B27, blocks lysis of porcine cells by several NK clones.[22] Transgenic expression of HLA-E and HLA-G on PAECs protects them from NK-cell-mediated xenogeneic cytotoxicity.[23,24] NK cells may also play a more direct role in rejection because NK activation by PAECs significantly augments the specific $CD4^+$ T cell response through the production of interferon-γ. We have observed that this response is partially dependent on the production of IL-12 (unpublished data).

Macrophages may also play an active role in the rejection of xenografts. It has been shown that human monocytes bind to the Galα1-3Gal antigen on porcine endothelium,[25] and macrophages have been found in the cellular infiltrate of rejected cardiac xenografts.[12] Cytokines such as interferon-γ and tumor necrosis factor-α augment monocyte adhesion and, therefore, may play a central role in cellular rejection.[26]

Infection

As mentioned above, another area of interest is the understanding of potential infectious agents and the development of ways to overcome possible transmission of animal pathogens to human cells. It is feared that the introduction of these organisms into an immunologically naïve population would be catastrophic.[27] Furthermore, genome-level incorporation could lead to the vertical transmission of such agents. These organisms appear to be well tolerated by their natural hosts; however, infections in a human host may be fatal, with varying degrees of rapidity.

According to some researchers,[28,29] numerous exogenous infectious agents have been identified in pigs, and outlines have been established to eliminate them from donor populations. However, despite these programs, unrecognized or latent organisms may exist that could prove to be pathogenic in humans.[2,30] A human hepatitis-E-like virus and a torovirus have been discovered and are concerning for possible infectivity.[2]

The porcine endogenous retroviruses (PERVs) have received significant attention in the literature. In 1970, Armstrong et al.[30] used thin-section electron microscopy to study porcine kidney cell lines and identified C-type virus particles that we now know to be PERVs. The PERV family is related to

a group of oncoviruses found in cats, mice, and gibbon apes[31] that have been associated with leukemia in their hosts. The effect of PERVs on human cells is unknown, but would depend on the site of their integration into the genome and the nature of their long terminal repeats.[32]

We know now through the work of Patience et al.[33] and others[34] that PERVs derived from the porcine cell line PK15 are able to infect human cells. Although human serum is effective at neutralizing virions shed from porcine cells, virions subsequently shed from infected recipient cells are relatively immune to human serum and complement-mediated destruction. Expression of virus capable of infecting human cells has recently been found in both porcine peripheral blood mononuclear cells and endothelial cells.[35,36]

In an analysis of the human $CD8^+$ T cell response to porcine antigens, we eluted peptides bound to SLA class I molecules from porcine spleens. Interestingly, sequencing of 2 peptides demonstrated that the peptides were derived from a retroviral transactivating regulatory protein (TAT) and an endogenous transcription factor. Furthermore, a synthetic peptide matching the TAT sequence increased lysis of peptide-depleted PAECs by xenoreactive $CD8^+$ T cells generated in vitro. This suggests that SLA class I expressing cells present peptides derived from PERVs and may be an important component in the xenoantigen repertoire recognized by human $CD8^+$ T cells. The implications of this finding as they relate to the immune response to xenotransplantation are currently being analyzed.

Numerous patients have been exposed to porcine tissues for therapeutic indications such as pancreatic islet xenografting and extracorporeal liver or kidney perfusions. Yet, despite the in vitro studies demonstrating infectivity of human cells, researchers have been unable to demonstrate PERV infections in these individuals.[37-40] It is unclear why individuals exposed to the virus do not develop infection, but it may be related to naturally occurring antibodies against the Galα1-3Gal epitope.

Conclusion

As organ transplant waiting lists grow and median times on the list lengthen, we must look at new ways to address the critical shortage of human organs. Although the barriers to clinical xenotransplantation are still great, basic research continues to make progress toward the goal of the feasible use of xenografts. The problem of humoral immunity has been well researched, and possible solutions have been investigated. Understanding the mechanisms of cellular immunity should now become a priority. By fully understanding these complex processes, we may begin to target specific aspects to prevent rejection. As clinical xenotransplantation becomes more a reality, focus will shift to the long-term consequences, specifically as they relate to xenozoonoses. Whether porcine-specific viruses will pose a true threat to human beings remains to be determined.

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