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Challenges and potentials of xenotransplantation

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Xenotransplantation refers to the transplantation of living cells, tissues, or organs from individuals of species into another species. Among the various fields of transplantation, none has sparked greater excitement and none greater controversy than xenotransplantation. This chapter will consider the various applications that have been proposed for xenotransplantation, as it is these from which the excitement derives. The chapter addresses the hurdles that prevent the application of xenotransplantation for the treatment of disease today, particularly those hurdles stemming from the immune response of the recipient to the graft. It is from these hurdles and the possibility that xenografts might serve as a vehicle for transfer of infectious organisms between species that the controversy derives.

APPLICATIONS FOR XENOTRANSPLANTATION

Although various types of xenografts were contemplated and even tried during all of history, xenotransplantation was first undertaken in the early years of the 20th century for the treatment of renal failure. Experimental surgeons had recently devised the vascular anastomosis as a way of connecting the cut end of blood vessels, and that advance created the field of vascular surgery. The vascular anastomosis would allow the repair of traumatic wounds and the penetration of surgery deeper into body cavities. However, those who developed the procedure realized the vascular anastomosis might also prove to be the critical technical advance needed to replace a sick organ with a healthy one, i.e., for organ transplantation.[1] As exciting as the prospect of organ replacement seemed to be, it was not clear then how one could obtain an organ from a human for transplantation. It was reasoned then that since some cellular components of the kidney and other organs often remain alive long after a person is deceased, harvesting a kidney from a cadaver would be unethical. Because of this concern, the first application of the vascular anastomosis to replacement of organ function was conducted using animals – swine and sheep – as a source of organs instead of humans.

The most important reason for interest in xenotransplantation today is to provide a plentiful source of organs, in lieu of human organs, for transplantation. By some estimates, the number of human organs available for transplantation equals as little as 5% of the number needed.[2] In the case of the kidney and liver, this shortage may be blunted by the use of living donors. However, for reasons discussed below, the demand for organ transplantation may soon increase further, and dramatically so.

Today, organ transplantation is mainly undertaken to treat severe failure of the kidneys, liver, heart, and lungs. As advances in public health and medicine allow many to live to an advanced age, the prevalence of type 2 diabetes and cardiovascular disease will increase, as will the demand for transplantation. The demand for transplantation could increase further as advances in molecular diagnostics, genomics, and proteomics make it possible to detect tumors and other lethal diseases before they are clinically apparent. The diagnosis of such disease will spark interest in using transplantation to pre-empt these conditions or to spare the patient from the risk of waiting until the tumor can be localized.

Xenotransplantation may even be preferred in some circumstances over human-to-human transplantation. When a virus causes organ failure, xenotransplantation might be preferred to avoid infection of the transplanted organ. This approach has already been attempted for treatment of cirrhosis caused by hepatitis virus and loss of immune competence caused by human immunodeficiency virus (HIV). A xenograft (or at least a xenogeneic organ) might be used as a temporary measure to preserve life or health until healing occurs (or until a human organ becomes available). Thus, the blood of subjects with fulminant hepatic failure has been through swine livers as a way of improving the subject's condition so that the patient could undergo allotransplantation. Xenografts might be used to deliver genes or gene products for complex metabolic pathways, exploiting the ability to express heterologous genes at high levels in genetically engineered animals. Xenografts (human-to-animal) have even been advanced as systems that might coax the differentiation of stem cells into functional tissues and organs.[3]

Unfortunately, despite the many potential uses and the long history of interest in xenotransplantation, daunting biological barriers presently prevent widespread application of xenotransplantation. These barriers include the graft injury caused by the immune response of the recipient against the graft, physiological incompatibilities between the transplant

and the recipient, and the possibility that the graft might transfer an infectious organism to the recipient. We shall consider all of these barriers, particularly the first.

THE SOURCE OF XENOGRAFTS

Ideally, xenografts should be obtained from species that are genetically similar to the recipient. Genetic proximity would tend to minimize the immunological barriers to transplantation and might improve the capacity of the graft to function. For human recipients, that ideal source would be nonhuman primates. Table 81.1 lists the outcome of some experimental trials of clinical xenotransplantation. Renal xenografts from chimpanzees functioned up to 9 months in human subjects with renal failure and undoubtedly far better results could be achieved today.

Despite the advantages of minimizing the genetic disparity between the sources and the recipients of xenografts, nonhuman primates are not generally viewed as useful sources of clinical xenografts. Nonhuman primates are not sufficiently numerous and the most abundant are too small in size to provide the organs and tissues needed for clinical xenotransplantation. Also of great concern is that nonhuman primates may harbor infectious agents, particularly viruses, such as herpes B and simian retroviruses, that are incompletely characterized and potentially lethal in humans.

Instead of nonhuman primates, the species that has received the most attention as a potential source of clinical xenografts is the pig. Pigs are available in large numbers and certainly would fulfill any conceivable need for organs and tissues. Pigs are of appropriate size to provide functioning organs for adults. And, because they are born in litters, pigs can be genetically engineered and bred with relative ease. Thus, both transgenic and "knockout" pigs have been developed for xenotransplantation, as discussed below. Finally, the infectious agents potentially carried by pigs are well characterized and, with the exception of an endogenous retrovirus, can be eliminated by scrupulous breeding and housing programs.

KEY CONCEPTS

- Xenografts excite both innate and adaptive immune responses
- The innate response includes xenoreactive natural antibodies, complement, and natural killer cells
- The adaptive immune response includes T-cell responses to peptides derived from many xenogeneic proteins plus major histocompatibility complex
- The adaptive humoral response includes antibodies against many xenogeneic antigens
- The biological outcome of a xenograft depends on the type of graft
- Organ xenografts are susceptible to vascular rejection and cellular rejection and are protected by accommodation
- Tissue and cell xenografts are susceptible mainly to cellular rejection

THE IMMUNOLOGICAL BARRIER TO XENOTRANSPLANTATION

As might be expected, many facets of innate and elicited immunity target xenografts. Thus, xenografts engender powerful responses by complement, phagocytic cells, natural killer (NK) cells, antibodies, and T cells. The intensity of these responses was once thought to be a direct function of the phylogenetic distance between the source of the graft and the recipient. However, many of the responses to xenotransplantation are not direct functions of genetic differences, and indeed some of the most daunting responses, particularly by antibodies and complement, can be powerfully exerted between serum and tissue constituents from animal species that are relatively closely related. In the sections that follow, we discuss the components of innate and elicited immunity that recognize and injure xenografts. Following, we shall consider how these components actually injure grafts.

INNATE IMMUNE RESPONSE TO XENOTRANSPLANTATION

Complement

Complement poses the greatest barrier to xenotransplantation, at least for organ xenografts. Once activated in a xenogeneic organ graft, complement can destroy the function and integrity of the graft within a few minutes to hours. This dramatic reaction, called hyperacute rejection, has been used over the years to test for complement inhibitors.

Complement of humans and other mammals can be activated in a xenograft by the alternative pathway or by the classical pathway (Chapter 20). The alternative pathway of complement is regulated on homologous surfaces, but not on some heterologous surfaces, by factor H, which inhibits the association of C3b with factor B. Activation of the alternative pathway of complement appears to provide a primary barrier to xenotransplants between many combinations of donor and recipient species. In some settings, however, for example in transplants of porcine organs into baboons or humans, the alternative pathway of complement is not spontaneously activated, presumably because factor H is functional across these species. In some of these combinations of source and recipient species, xenoreactive natural antibodies, as discussed below, activate complement.

Regardless of which mechanism initiates activation of complement, the kinetics and extent of complement activation is increased due to the lesser ability of complement regulatory proteins to control heterologous complement, a condition called homologous restriction.[4] Some recent studies have challenged the importance of homologous restriction; however, the observation that xenografts from transgenic animals express very low levels of complement regulatory proteins of the recipient and avoid the most severe types of complement-mediated injury[5] provides compelling support for the biological importance of homologous restriction and suggests it is limiting for xenotransplantation.

The devising of effective approaches to preventing or controlling activation of complement has been seen for many years as key to the successful application of xenotransplantation. In experimental models, agents such as cobra venom factor, which activates the alternative pathway of complement, and soluble complement receptor type 1 have been found

Table 81.1 Some clinical attempts at xenotransplantation

Year	Donor	Organ	Maximum survival
1906	Pig	Kidney	2 days
1964	Chimpanzee	Kidney	9 months
1964	Baboon	Kidney	60 days
1984	Baboon	Kidney	20 days
1992	Baboon	Liver	70 days

to provide sufficient protection to allow survival of organ xenografts for periods of days to weeks. Despite the obvious advantages of administering complement inhibitors, this approach has several important limitations. First, the action of complement inhibitors is transient, whereas complement activation persists. Second, systemic inhibition of complement compromises host defense against infections. The alternative, and now standard, approach to controlling activation of complement is to express complement regulatory proteins of the recipient as the product of transgenes in the would-be source of xenografts.[6] For example, expression of human decay-accelerating factor, CD59, and membrane cofactor protein in transgenic pigs has led to prolonged survival of organs from those pigs transplanted into nonhuman primates.[5,7]

Xenoreactive natural antibodies

All vertebrates capable of producing antibodies have at least some natural antibodies. These antibodies are called 'natural' antibodies because they are produced without a known history of sensitization with the corresponding antigen. Natural antibodies include polyreactive antibodies, which bind to many different and disparate types of antigens, and monoreactive natural antibodies, which mainly recognize one or a few saccharides. The best-known monoreactive natural antibodies are the isohemagglutinins, which recognize the blood group A and B antigens.

Xenoreactive natural antibodies (natural antibodies that recognize the cells of foreign species) have been known for decades.[8] Xenoreactive antibodies may be polyreactive or monoreactive. Xenoreactive polyreactive antibodies, like other polyreactive antibodies, are thought to be made by B1 B cells. Whether xenoreactive polyreactive antibodies activate complement or exert other effector functions and cause disease is uncertain. On the other hand, monoreactive xenoreactive antibodies are thought to contribute an important barrier to xenotransplantation.

The best-characterized xenoreactive antibodies are antibodies specific for Galα1-3Gal. Galα1-3Gal is produced by the action of α1,3-galactosyl transferase on growing oligosaccharide chains. Functional α1,3-galactosyl transferase is produced by all lower mammals and by New World monkeys. Humans, apes, and Old World monkeys do not produce a functional enzyme, and hence they make no Galα1-3Gal. Species such as human that make no Gala1-3Gal produce natural antibodies, sometimes in large amounts, specific for that sugar. Antibodies specific for Galα1-3Gal are monoreactive and have functional properties like antibodies specific for the A and B blood groups.

Therapeutic strategies for limiting the impact of xenoreactive antibodies have focused on: (1) immunoabsorption of antibodies; (2) inhibition of production by immunosuppressive drugs or induction of tolerance; (3) the generation of pigs with targeted disruption of the α1,3-galactosyl transferase; and (4) various combinations of these approaches. Depletion of xenoreactive antibodies by passage of blood through xenogeneic organs or through immunoaffinity columns has helped to demonstrate that antibodies induce much of the activation of complement in xenogeneic organ grafts and has provided some prolongation of graft survival. On the other hand, no regimen of immunosuppressive drugs yet tested has effectively inhibited production of natural antibodies (cyclosporine, leflunamide, and some other agents have helped to a varying extent to limit production of elicited antibodies). Various strategies have been devised to induce tolerance to Galα1-3Gal. For example, α1,3-galactosyl transferase has been expressed in bone marrow cells of a would-be recipient, and this has apparently led to tolerance to Galα1-3Gal in rodents. However, no approach to induction of tolerance tested to date, including the induction of mixed hematopoietic chimerism,[9] has yielded an enduring state of tolerance to Galα1-3Gal in nonhuman primates. Rather, the most effective way to deal with natural anti-Galα1-3Gal antibodies is the production of pigs with targeted disruption of α1,3-galactosyl transferase.[10] Organs from such pigs contain little Galα1-3Gal and survive for prolonged periods after transplantation into nonhuman primates.[11,12] However, the recipients of organs from the Galα1-3Gal-deficient pigs were always treated with other manipulations, so it is difficult to know how much of the prolongation of survival is owed only to the absence of Galα1-3Gal.[13]

Natural killer cells

Xenogeneic cells are also recognized by NK cells and recognition leads to cytotoxicity *in vitro*. The mechanisms that give rise to cytotoxicity are thought to include the following: (1) failure of major histocompatibility complex (MHC) class I on the xenogeneic target to interact with killer inhibitory receptors of NK cells; (2) stimulation of FcγII receptors on NK cells by xenoreactive immunoglobulin G (IgG) bound to xenogeneic cells; and (3) stimulation of lectin receptors on NK cells by saccharides such as Galα1-3Gal on the xenogeneic target cells and activation of other cytotoxicity receptors. To what extent NK cells damage organ transplants is uncertain, as immunodeficient animals with normal NK activity can accept allografts and xenografts. However, some recent studies suggest that NK cells can induce vascular injury over a period of time, especially in xenografts. NK cells might also promote antigen presentation and, thus, elicit immune responses directed against the xenograft; these responses appear to be a main limitation of application of xenotransplantation.

Perhaps because of uncertainties about the importance of NK cells in the barrier to xenotransplantation, therapeutic strategies for inhibiting these cells have been discussed but so far have not been advanced. Among the considerations are transgenic expression of human leukocyte antigen (HLA)-E or other stimulators of inhibitory receptors.

Inflammation and coagulation as innate barriers to xenotransplantation

Besides those components generally viewed as elements of the innate immune system, phagocytes (neutrophils and macrophages), platelets, and the coagulation system can recognize and react directly with xenografts. Of course, these elements can also be recruited by innate and adaptive immune responses. Whether these cells and pathways cause greater harm by direct action on xenografts or as effectors recruited by immune reactions has not been tested. However, since inhibition of innate and elicited immunity largely prevents acute destruction of xenografts, one suspects that these elements mainly serve an accessory capacity.

ELICITED IMMUNE RESPONSES

T cells

T cells clearly recognize and destroy xenografts since cellular xenografts can survive in nude mice, but not in wild-type mice. To which extent and by what mechanism T cells recognize xenogeneic cells, however, have been matters of controversy. T-cell responses to xenogeneic cells appear to be defective when evaluated *in vitro* because cytokines and co-stimulatory molecules produced by xenogeneic antigen-presenting cells (APCs) act poorly on responding T cells. However, xenogenic APCs can present foreign MHC antigen to T cells, presumably through the use of alternative co-stimulatory pathways and cross-reactive properties of T-cell receptors. T cells may also respond *in vitro* to foreign peptides presented by self-APC. Although the nature and intensity of T-cell responses to xenotransplantation are still not completely known, the response is likely to be at least as intense as the response to allotransplantation, owing to the diverse set of antigenic peptides, an amplifying effect of humoral immunity and inflammation on cellular immune responses, and defective immune regulation.[14]

Elicited antibodies

Xenogeneic cells and tissues elicit powerful and diverse antibody responses. These responses, like other T-cell-dependent responses, are controlled to some extent by immunosuppressive therapy. The elicited immune response has been seen as the critical barrier to transplantation of xenogeneic organs, yet little is known about the specificity. Xenotransplantation does lead to the production of a large amount of anti-Galα1-3Gal antibodies, consisting of a greater fraction of IgG and exhibiting higher affinity than natural anti-Galα1-3Gal antibodies. However, whether these antibodies result from class switch recombination and affinity maturation in B cells producing natural Ig or the activation of novel clones is uncertain. Because production of anti-Galα1-3Gal antibodies or their impact on the graft might be controlled as described above, the elicited antibodies of greater importance are probably T-cell-dependent antibodies specific for other antigens.[15] Unfortunately, little is known about the specificity of the antibodies; however, these antibodies are now seen as another important barrier to successful transplantation of xenogeneic organs.

IMPACT OF IMMUNE RESPONSES ON THE XENOGRAFTS

If xenotransplantation excites nearly every facet of innate and adaptive immunity, the impact of an immune response is determined not so much by the intensity or diversity of the response as by the type of graft implanted: cell, tissue, or organ. The type of graft determines the impact of the immune response because it determines the means by which the graft receives its supply of blood, and it is the vasculature of grafts that is most vulnerable to immune-mediated injury. Figure 81.1 depicts the various responses observed following xenotransplantation of organs, tissues, and cells. Notice that organs are susceptible to various types of rejection that are not observed in cell and tissue grafts. These types of rejection focus predominantly on the vasculature of the graft.

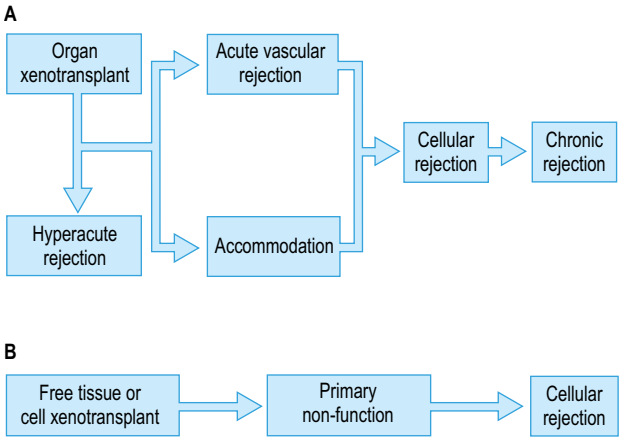

Fig. 81.1 Biological outcome of xenografts. (**A**) The outcome of organ xenotransplants. Organ xenografts are subject to vascular types of rejection, including hyperacute, acute vascular, and chronic rejection. Vascular rejection, particularly hyperacute and acute vascular rejection, are caused by the binding of antibodies and activation of complement of the recipient on xenogeneic blood vessels. Having blood vessels originating with the recipient, cell and tissue xenografts are not subject to this type of problem. Accommodation refers to acquired resistance to injury. Organ xenografts are also susceptible to cellular rejection. (**B**) The outcome of free tissue and cell xenotransplants. Cell and tissue xenografts derive their blood supply through the in-growth of blood vessels of the recipient. Since the blood vessels of these grafts are constructed from cells of the recipient, antibodies of the recipient do not generally bind to the blood vessels, and hence vascular diseases of organ grafts such as hyperacute and acute vascular rejection are not observed. Free tissue and cell xenografts are mainly subject to injury by T cells that have the ability to migrate effectively through blood vessels walls, causing primary nonfunction and cellular rejection.

HYPERACUTE REJECTION

Hyperacute rejection refers to the rejection of an organ graft within 24 hours of reperfusion; it is arguably the most severe and violent immunological reaction as it reflects the loss of graft function and destruction of the organ within a period of hours. Hyperacute rejection begins within minutes of the perfusion of a newly transplanted organ and is characterized by formation of platelet thrombi and bleeding into the graft. Organs transplanted between disparate species are especially susceptible to hyperacute rejection.

The development of hyperacute rejection depends absolutely on activation of complement in an organ graft. In some combinations of organ and recipient species, complement activation is initiated directly through the alternative pathway, owing presumably to species-specific function of factor H. This type of hyperacute rejection is especially severe and resistant to therapy, perhaps because C3b attaches simultaneously to many available sites on blood vessel walls. In clinically relevant combinations of organ and recipient (e.g., swine organs transplanted in higher primates), complement activation is mainly initiated by the classical pathway, owing the binding of xenoreactive antibodies and involvement of the alternative pathway is secondary. In this setting, the kinetics and extent of complement activation are functions of mainly antibody–antigen interaction.

Hyperacute rejection appears to reflect a loss of endothelial cell function. This loss is triggered by terminal complement complexes inserted into endothelial cell membranes. The rate of complement activation appears particularly important, as measures that slow the formation of terminal complexes, such as expression of very low levels of decay-accelerating factor and/or CD59 from the recipient species in blood vessels of the graft may prevent the disease.[5]

Hyperacute rejection can be prevented by any means that hinders activation of complement in the transplant. In pig-to-primate xenografts, such means include the depletion of xenoreactive antibodies from the circulation of the recipient, elimination by gene targeting or other means of the antigen they recognize, and the inhibition of complement reactions, such as through expression as the product of transgenes of complement regulatory proteins of the recipient species.

ACUTE VASCULAR REJECTION

If hyperacute rejection does not occur or if it is prevented, a xenografted organ is susceptible to acute vascular rejection. Acute vascular rejection emerges over a period of days to weeks and is characterized by endothelial swelling, focal ischemia, and intravascular coagulation. Acute vascular rejection, sometimes called delayed xenograft rejection or acute humoral rejection, causes destruction of a xenograft over a period of days to weeks and is now widely seen as a third major hurdle to the clinical application of organ xenotransplantation.[14]

Acute vascular rejection appears to be caused mainly by the action over hours to days of antibodies of the recipient directed against the graft. Besides antibodies, other factors, including macrophages, platelets, and NK cells, have been implicated in this disease. However, most transplant physicians are so persuaded about the importance of antibodies that when this type of rejection occurs in an allograft it is often referred to as 'antibody-mediated' rejection.

Binding of antibodies to blood vessels in a graft is thought to cause acute vascular rejection (if it does not cause hyperacute rejection) by activating endothelium. In some cases at least, activation of endothelium depends on activation of complement and particularly the insertion of sublytic amounts of terminal complement complexes in endothelial cells. Terminal complement complexes activate the interleukin (IL)-1α gene, leading to production of that cytokine; under some conditions, it is that cytokine that determines the subsequent fate (activation versus nonactivation) of endothelium and hence of the graft. Endothelial cell activation changes the posture of blood vessels from anti-coagulant to procoagulant and from anti-inflammatory to proinflammatory.

Several approaches have been pursued in efforts to prevent or treat acute vascular rejection. To the extent that anti-Galα1-3Gal antibodies trigger acute vascular rejection, the induction of immunological tolerance to Galα1-3Gal or the elimination of that saccharide from transplants might prevent the initiation of that process. However, neither tolerance induced by presently available means nor the elimination of Galα1-3Gal can prevent acute vascular rejection of xenografts.[12, 13] In these cases, antibodies elicited against antigens other than Galα1-3Gal appear to incite rejection. Perhaps tolerance might be induced to a broader spectrum of antigens by transplantation of hematopoietic stem cells of the donor species into the recipient. Still another approach to preventing acute vascular rejection of xenotransplants, however, may involve the induction of accommodation.

ACCOMMODATION

Accommodation is an acquired resistance to humoral injury and acute vascular rejection of an organ graft.[6, 16] Accommodation was first observed in the transplantation of kidneys across blood group A and B barriers when transient removal of anti-blood group antibodies from the recipients of the transplants was followed by prolonged function of the transplants after the return of the antibodies to the circulation. Accommodation has been observed in rodent models of xenotransplantation and in porcine organs transplanted into baboons where the organs express human complement regulatory proteins and the xenoreactive antibodies are temporarily depleted from the circulation of the xenograft recipient. Accommodation may exemplify a broader response in which cells exhibit reversal of noxious pathways.[16] The development of accommodation may be important for the successful engraftment of xenogeneic organs because these organs contain numerous antigens that could evoke humoral immune responses.

We originally postulated that accommodation may reflect one or more of three changes following organ transplantation: (1) a change in the nature of xenoreactive antibodies; (2) a change in the antigen-impairing antibody binding; and (3) induction of cellular resistance to humoral injury.[6] Most evidence would presently point to acquired resistance to injury as being central to accommodation. Accommodation in both rodents and pig-to-primate xenografts appears to be associated with expression of various anti-apoptotic proteins and heme oxygenase-1. In other biological systems, accommodation may require the AKT and PI3 kinase system. Which gene(s) and signaling pathways actually bring about accommodation and which are simply needed for cell survival, but not accommodation *per se,* is still uncertain.

CHRONIC REJECTION

Whether or not, and to what frequency, chronic rejection would occur in a vascularized xenograft is uncertain because of the difficulties in overcoming acute vascular rejection. Clearly, ongoing production

of anti-donor antibodies might be expected to cause chronic rejection of xenografts. If complement activation on endothelial cells or smooth-muscle cells induces proliferation of the cells and the development of chronic lesions, one might also anticipate such lesions in vascularized xenografts. Whether cell-mediated immunity would cause chronic rejection of xenografts, as it does in allografts, is unknown. On the other hand, to the extent that nonimmunologic causes of chronic rejection, such as preservation injury and infection, contribute to chronic rejection, that problem might be less in an organ xenograft.

CELLULAR REJECTION

Organ xenografts are subject to cellular rejection more or less like what is observed in organ allografts. The rejection of organ xenografts, like the rejection of organ allografts, presumably targets foreign major histocompatibility antigens. However, unlike allografts, practically all of the non-MHC proteins in xenografts are immunogenic. Above we discussed the several distinct aspects of this response. Here it can be said that cellular immune responses to xenografts are not necessarily limiting and are apparently subject to control by the same therapeutic agents as cellular immune responses to allografts. Some evidence does suggest that the response to xenografts may be especially susceptible to therapeutics aimed at CD4 T cells.

COAGULATION AND THROMBOSIS IN XENOTRANSPLANTATION

Organ xenografts are plagued by coagulation and thrombosis. Thrombosis occurs in acute vascular rejection and may occur independently of rejection, owing to defective control of thrombin generation and hemostasis on xenogeneic endothelium. As two potential mechanisms for the latter, porcine von Willebrand factor spontaneously aggregates human platelets and porcine thrombin might interact inefficiently with human thrombomodulin.

CLINICAL RELEVANCE

Xenografts could provide an abundant source of organs, tissues, and cells for transplantation

Xenografts might evade recurrence of viral or immunologic disease

Some day, human cells, tissue, or organs might actually be grown in animals for transfer later into humans

Xenografts provide excellent models for elucidating mechanisms of vascular disease and accommodation and for testing the efficacy of modifiers of innate and adaptive immune responses

REJECTION OF CELL AND TISSUE XENOGRAFTS

Although cell and tissue xenografts incite humoral immune responses, unlike organ xenografts, cell and tissue xenografts are not subject to devastating vascular rejection. Cell and tissue xenografts are not susceptible to vascular rejection because the blood vessels of those grafts grow in from the recipient and thus consist of cells to which the recipient is tolerant. Consistent with this concept, cell and tissue xenografts appear to be undisturbed by high levels of antibodies in the recipient specific for the grafted cells. On the other hand, cell and tissue xenografts are susceptible, perhaps quite susceptible, to primary nonfunction and to cellular rejection (Fig. 81.1B).[14] Both primary nonfunction and cellular rejection may be mediated by T cells; however, NK cells might also have importance, for reasons discussed above.

INFECTIOUS DISEASE AS A BARRIER TO XENOTRANSPLANTATION

If the immunological response of the recipient to a xenograft poses the most difficult biological barrier to xenotransplantation, the potential transfer of infectious organisms from the graft to the recipient may imperil the health of the community. While many microbial organisms infect one or only a few related species, some, such as influenza and severe acute respiratory syndrome (SARS), can pass between disparate species and then more broadly, with potentially devastating consequences.

In principle, the transfer of infections from a graft to the recipient should be less of a problem in xenotransplantation than in allotransplantation. After all, one can characterize infectious agents present in an animal source and potentially eliminate those agents by breeding and special housing conditions. Further, because the microbial organisms present in a source of xenografts are potentially known, the benefits of transplantation can be weighed against the risks of infection. Further, if the source of xenografts is a species such as the pig, which has been in close contact with humans for centuries, then the risks of zoonotic disease are known.

One exception to this sanguine view of trans-species infection in xenotransplantation is the possibility that pigs might have an endogenous retrovirus that could pass into the human, cause disease, and eventuate human-to-human infections. Endogenous retroviruses are broadly represented in the species (and indeed they are transmitted vertically) and hence cannot be easily eliminated by breeding. The porcine endogenous retrovirus (PERV) present in various forms in all strains of pigs is such an agent. PERV from porcine cell lines and from activated porcine cells can infect human cells in culture. However, extensive surveys of humans who received experimental xenografts of various types or who were temporarily exposed to porcine organs and blood products have failed to provide any evidence that PERV can pass from pig organs or tissues to human cells *in vivo*.[17] However, the analysis of chimeric pigs harboring human hematopoietic cells revealed PERV could pass from swine into human cells if the swine and human cells fuse spontaneously.[18] Indeed, cell fusion provides a potential mechanism for retroviral transfer and recombination between species (Fig. 81.2). Passage of PERV into

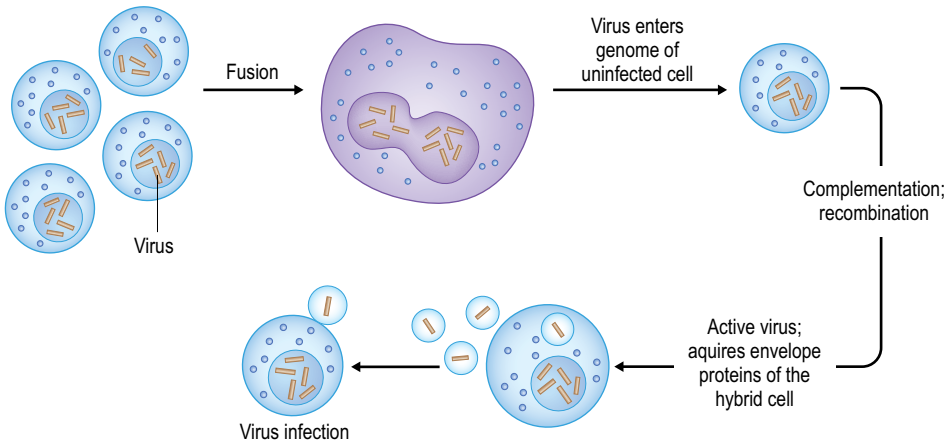

Fig. 81.2 Transmission of virus by cell fusion. Cell fusion may facilitate transmission of viruses and contribute to emergence of novel viruses. Fusion of a cell infected with a virus with an uninfected cell allows the virus (dark black bar) from infected cell (blue) to enter the genome of uninfected cell (red) by nuclear fusion. When the virus is activated and buds from the 'infected' cell, it acquires surface proteins from the uninfected fusion partner (red) and is thus capable of infecting other similar cells by viral entry. When nuclear fusion allows a virus of one species to enter the genome of another species, mutation and translocation of chromosomes may facilitate the generation of novel viruses. (Adapted from Ogle BM, Cascalho M, Platt JL. Biological implications of cell fusion. Nat Rev Mol Cell Biol 2005; 6: 567–575.)

human cells by cell fusion would have been missed in previous surveys because the conclusion that infection had not occurred depended on quantitative or semiquantitative polymerase chain reaction (PCR) or assay of swine–human chimerism, and when cells fuse, some genes, potentially those assayed in the PCR reaction, are lost. Thus, the question of PERV transfer into humans remains to be answered.

One other implication of xenotransplantation for host defense pertains to the control of intracellular infections by cell-mediated immunity. Since cell-mediated immunity depends to a certain extent on T-cell responses that are more or less MHC-restricted, the recipient might not control viruses and other intracellular organisms that invade the graft. Thus, the factors, such as MHC restriction, incompatibility of cytokines and cell dhesion molecules that impair T-cell responses to xenogeneic cells, discussed above, might have the greatest impact on host defense. At present, this limitation is theoretical; however, some who conduct experimental transplants have noticed that viruses such as CMV may engender more severe or more complicated problems in the recipients of xenografts than in the recipients of allografts.

CONCLUDING REMARKS

The immunology of xenotransplantation is a subject of medical interest. The application of transplantation for the treatment of human disease is significantly constrained by a shortage of human organs, and overcoming the immunological barriers to xenotransplantation would surely solve that problem. Whether xenotransplantation is applied for this purpose and to what extent will also depend on advances made in other technologies.[3, 19] One might imagine that cloning and new applications for stem cells could efface or even eliminate the demand for xenotransplantation. However, interest in xenotransplantation will likely persist, despite the failure to overcome the immunological barriers and the advance of alternative technologies. Such interest should stem from xenotransplantation as an experimental model. Thus, today, one can find no better or more rigorous model than a xenograft for testing the efficacy of therapeutics for the complement or coagulation systems. Furthermore, xenografts have provided very powerful models for elucidating the mechanism of complement-mediated injury, the pathophysiology of endothelial cell activation, and the biological phenomenon of accommodation.[6] Finally, xenografts have begun to teach unexpected lessons, such as the developmental implications of cell fusion and mechanisms of transfer of viruses between species.[18]

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