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CHAPTER

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Xenozoonoses: The Risk of Infection after Xenotransplantation

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OUTLINE

I. INTRODUCTION

Immunological and technical advances have led to tremendous increases in the number of people potentially able to benefit from allotransplantation. Ironically, it is the success of the field that has led to a renewed interest in xenotransplantation during the past several decades. To a large part, this has occurred because of the great scarcity of human organ and tissue donors. However, it has expanded to include the use of cells from animals into humans such as porcine islet cells for diabetes or extracorporeal perfusion of human blood through animal organs or cells. Similar to allotransplantation, issues regarding transmission of infections from the graft to the human recipient were brought up for consideration with these procedures in the 1990s (Michaels and Simmons, 1994; Chapman *et al.*, 1995; Hammel *et al.*, 1998; Fishman *et al.*, 1998). A risk for infection exists with the use of any biologic agent regardless of whether it is from a human or an animal source. Accordingly, transmission of infections from human organs, tissues, or cells is a well-recognized cause of disease after allotransplantation (Ison and Grossi, 2013; Green and Michaels, 2012). As the human graft shortage continues, newer cellular therapies are explored. Thus, attention continues to be given to the potential use of xenogeneic organs, tissues, or cells for human maladies through xenotransplantation. The potential for novel zoonotic infections to emerge because of xenotransplantation (xenozoonoses or xenosis) led to a debate on whether the field should be permitted to progress. This chapter reviews the issues of xenotransplantation related to infections from animals to humans. Lessons learned from infections with prior nonhuman primate xenotransplantation and human allotransplantation are used to help inform about risks with newer xenogeneic procedures. In addition, information on known zoonoses

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is reviewed to better develop constructs to decrease the hazard of infection with these novel procedures.

II. LESSONS FROM ALLOTRANSPLANTATION/HISTORICAL PERSPECTIVE

While the field of allotransplantation has advanced significantly over the years, infections remain a substantial cause of morbidity and mortality. The major risk factor for severe infections is the use of nonspecific immunosuppression to prevent rejection of the new graft. In xenotransplantation, where systemic immunosuppression is even more intense than in allotransplantation, risks of infection by commensal or opportunistic pathogens are significant. Sources of microbes can be from the recipient's endogenous flora, the environment, or organisms harbored within the donated organ, tissues, or cells (Ison and Grossi, 2013; Green and Michaels, 2012). The first two sources, the environment and the recipient's endogenous flora, are the same regardless of if a person undergoes an allo- or xenotransplant. These contributed to the deaths of five of six recipients that received a baboon or chimpanzee kidney xenotransplant in two separate series from the early 1960s (Reemtsma *et al*., 1964; Starzl *et al*., 1964). Similarly, the first baboon-to-human liver xenotransplant recipient died from aspergillus, an environmental pathogen after receiving aggressive immunosuppression (Starzl *et al*., 1993). A second recipient of a baboon liver xenotransplant also succumbed to infection, dying with multiorgan failure 26 days after transplantation largely due to sepsis from his endogenous intraabdominal bacteria secondary to an anastomotic leak (Starzl *et al*., 1994). These human clinical trials using animal organs identified infections that were caused by immunosuppression and surgical complications.

However, it is possible that the graft may lead to novel infections. Some donor-associated infections are often predictable. These agents are often maintained in a quiescent intracellular state and are asymptomatic in the donor. They can potentially be transmitted via the graft organ or the accompanying hematopoietic cells (Ison and Grossi, 2013; Green and Michaels, 2012; Michaels and Simmons, 1994). Examples include blood-borne pathogens such as hepatitis B virus (HBV), hepatitis C virus (HCV), and retroviruses, along with some herpesviruses and parasites. Similar classes of organisms are of concern with animal organ transplantation and are worth examining more fully. Human herpesviruses, in particular, human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV) are important donor-associated infections after allotransplantation. Their transmission from donors was first suspected by epidemiologic evidence and later confirmed using molecular techniques (Chou, 1986; Cen *et al*., 1991). Both HCMV and EBV cause more severe disease in naive hosts who undergo primary infection after transplantation (Rubin, 1990; Ho *et al*., 1985). In particular, seronegative recipients of organs from seropositive donors are at highest risk. However, even patients with previous immunity to HCMV or EBV can be reinfected with donor strains of these viruses (Chou, 1986; Cen *et al*., 1991; Rubin, 1990; Ho *et al*., 1985). Thus, it is unlikely that complete protection from analogous animal viruses after xenotransplantation will occur.

Some latent donor herpesviruses are not generally transmitted by transplantation. For example, herpes simplex virus (HSV) and varicella zoster virus (VZV) are latent in sensory ganglia and, as such, are not usually present in the blood or the transplanted graft. Consequently, they represent a very low risk of donor transmission and highlight the concept of relative risk for donor-associated infection based upon microbial tropism and individual properties of the organism. Other donor viruses outside the herpesvirus family can be transmitted. Blood-borne pathogens such as human immunodeficiency virus (HIV), HBV, and HCV have all been unintentionally transmitted after allotransplantation (Ison and Grossi, 2013; Green and Michaels, 2012; Pereira *et al*., 1991; Dummer *et al*., 1989). Usually, this happened when viruses were missed or screening tests were unavailable (Pereira *et al*., 1991; Dummer *et al*., 1989). However, even in the era of universal screening, transmission still occurs. Transmission of HIV from a single donor to four organ recipients and three of four bone marrow recipients was reported. The patient had had a negative HIV screening (Simonds *et al*., 1992; Ison *et al*., 2011). Retrospective analysis concluded that the donors were infected before a detectable antibody response could be mounted, emphasizing the inherent limitations of all screening tests. Even as improved screening tools become available, including nucleic acid testing (NAT), false-positive and false-negative results still occur (Humar *et al*., 2010).

Nonviral infections can also be transmitted during allotransplantation. Parasites such as *Toxoplasma gondii* are transmissible if the donor has organisms within the transplanted graft. *T. gondii* is an example of a donor-graft-specific infection. Naive heart transplant recipients are at highest risk because of the protozoa's tropism for cardiac muscle (Wreghitt *et al*., 1989; Campbell *et al*., 2006). On rare occasions, acute bacteremia or viremia is unrecognized in a donor and is transmitted to the new recipient. Prevention of disease relies largely on screening of donors. Except in the case of living related donation, donor screening is limited by substantial time constraints and the inability to retest serial samples. Prophylaxis and surveillance of recipients are important. The process should be dynamic in applying new protocols for screening and surveillance being developed.

Similarly, protocols for xenozoonoses will be invaluable to help in preventing infections from animals.

III. POTENTIAL MECHANISMS FOR CROSS-SPECIES INFECTIONS

Transmission of an animal pathogen could occur by several mechanisms (Michaels and Simmons, 1994; Chapman *et al*., 1995). First, an organism could be infectious to both the animal donor and the human recipient (*T. gondii* is an example). Second, animal viruses that are similar to human viruses, even if not currently known to be zoonotic, could infect humans with this novel access to human cells. This has been postulated for animal herpesviruses such as cytomegalovirus (CMV) and EBV (Michaels and Simmons, 1994; Michaels *et al*., 1994). Third, a nonpathogenic animal microbe could cause disease after xenotransplantation due to immunosuppression. Fourth, a viral recombination between animal and human viruses leading to a virulent recombinant strain is of concern. It is also possible that latent animal viruses present in the graft can reactivate, and without infecting the human, cause graft failure. Likewise, it is possible that human viruses may infect the animal graft and induce its failure. These latter concerns are particularly germane for xenotransplantation because the human recipient's immune system will not recognize porcine MHC receptors.

The concept of 'species specificity' deserves a more thorough discussion. If true, it is possible that xenotransplantation carries less risk of donor-transmitted infections than allotransplantation. However, examples of transmission of viruses that were considered to be species-specific can be found where the consequences are severe, such as with the herpesvirus family. The alpha herpesvirus of macaques, macacine herpesvirus 1 (B virus) is well established as a virus that is capable of being more pathogenic after crossing species lines (Artenstein *et al*., 1991; Cohen *et al*., 2002; Hilliard *et al*., 1989). Transmission to humans is rare, but usually lethal. Furthermore, and of public health concern, is documentation of a husband secondarily infecting his wife after he was infected by direct contact with infectious monkey secretions (Cohen *et al*., 2002). This example of a virus harmless in one species but causing more severe disease in another species with potential for secondary transmission is of major concern in xenotransplantation (Michaels and Simmons, 1994; Chapman *et al*., 1995; Hammel *et al*., 1998; Fishman *et al*., 1998; Institute of Medicine, 1996; Public Health Service, 2001; Kennedy, 1996; Sgroi *et al*., 2010; Archidiacono *et al*., 2010). Alpha herpesvirus such as macacine herpesvirus 1 is latent in nerve endings rather than in organ tissue or hematopoietic cells. Accordingly, similar to HSV, this alpha herpesvirus is anticipated to be of low risk for transmission via xenotransplantation; however, its disease severity makes any risk unacceptable.

During the past 20 years, only baboons and pigs have been used as source animals for attempted whole-organ xenotransplants, whereas a variety of animal sources including baboons, swine, rabbits, cows, sheep, and hamster have been used for tissue and cellular xenografts into humans (Sgroi *et al*., 2010). While nonhuman primates are no longer used as a source, they are still used as recipients in nonhuman experimental xenotransplant models; thus, lessons learned from when they were used are still valuable. Most information on potential pathogens is available for baboons and swine. Neither baboons nor swine harbor macacine herpesvirus 1, but they do have analogous alphaherpesviruses, Simian agent 8 (cercopithecine herpesvirus 2) (SA8) and pseudorabies, respectively (Michaels *et al*., 1994). Thus far, active surveillance has not found transmission of SA8 to humans. Pseudorabies can cause fatal disease in sheep, dogs, and cattle but has not been proven to be infectious to nonhuman primates (Sawitzky, 1997). However, an anecdotal report noted three immunocompetent humans with transient fever, weakness, and neurologic abnormalities to test positive for pseudorabies antibodies suggesting transmission between swine and humans (Archidiacono *et al*., 2010).

As noted, members of the alpha herpesvirus family show that infections across species' lines can be dangerous, but these viruses are unlikely to be easily transmitted. However, both baboons and swine harbor beta and gammaherpesviruses that are likely to be in tissues, cells, or organs (Michaels *et al*., 1994; Edington *et al*., 1988; Falk, 1976).

Transmission of CMV or EBV between disparate species has been suggested. The Towne strain of human CMV replicates in cultures of chimpanzee skin fibroblasts and baboon CMV replicates in human fibroblasts (Perot *et al*., 1992; Michaels *et al*., 1997). In addition, neurologic disease in two humans has been attributed to primate CMV (Huang *et al*., 1978; Charamella *et al*., 1973; Martin *et al*., 1994, 1995). In the first case, the Colburn CMV strain was reportedly isolated from a brain biopsy of an encephalopathic child and was homologous to African green monkey CMV (strain GR2757) (Huang *et al*., 1978; Charamella *et al*., 1973). In the second case, an African green monkey-like CMV was repeatedly isolated from a woman diagnosed with chronic fatigue syndrome (Martin *et al*., 1994, 1995). Both cases suggest potential transmission of a simian CMV to humans, but neither provides evidence for how the transmission may have transpired. More direct implications for xenotransplantation were found with the isolation of baboon CMV from a blood specimen of a recipient of a baboon liver 1 month after transplantation but not subsequently (Michaels *et al*., 2001). Swine CMV has been less extensively investigated; thus far, it has not grown

in human cell lines *in vitro*. Few studies on cross-species transmission of gammaherpesviruses such as EBV are available, although it has long been known that human EBV is able to infect marmoset lymphocytes in the laboratory (Miller, 1990). Our studies have also found variable cross-reactivity of antibody tests directed against human EBV antigens with antibodies found in baboons. Commercial tests for EBV viral capsid antigen found a high seropositivity rate in a baboon colony, whereas the majority of paired specimens were negative when tested for antibody against EBV nuclear antigen (EBNA). This finding may be related to differences in the conservation of some sites of gammaherpesviruses (Falk *et al.*, 1976).

Human organs, blood, and tissues have been vehicles for transmitting retroviruses, such as HIV. Retroviruses are often species restricted, but similar to herpesviruses, can cross species barriers. For example, simian immunodeficiency virus (SIV) appears to be benign in its natural host, the African green monkey, but progresses to an acquired immunodeficiency syndrome (AIDS)-like disease when inoculated into macaques (Benveniste *et al.*, 1988). Transmission is variable in other nonhuman primates. SIV and HIV type 2 are genetically similar (Benveniste *et al.*, 1988). Probable transmission to two humans who were exposed to SIV has been documented (Khabbaz *et al.*, 1992, 1994). One individual remained asymptomatic and gradually lost antibody against SIV over a 2-year period. The other person had SIV isolated from peripheral blood cells documenting an ongoing active infection albeit without clinical symptoms (Khabbaz *et al.*, 1994). SIV strains of chimpanzees have been identified as the origin of HIV type 1 and responsible for the human AIDS epidemic (Gao *et al.*, 1999). Little is however known about lentiviruses in pigs.

Retroviruses other than lentiviruses may be transmissible during xenotransplantation. It is particularly problematic when using nonhuman primates. Examples include simian T-lymphotropic virus (STLV) which is an oncogenic retrovirus found in many nonhuman primate populations. Genetically, STLV has homologous sequences with human T-lymphotropic virus, type 1 (HTLV). HTLV has been associated with leukemia in humans (Homma *et al.*, 1984). Foamy viruses are another class of retroviruses found in nonhuman primates named for a foamy cytopathic effect that was found in primary monkey cell lines that are used in virology laboratories. Surveillance of workers with occupational exposure to nonhuman primates have found several to be infected with foamy virus although no disease has been noted (Schweizer *et al.*, 1997; Anonymous, 1997). The exact timing of infection could not be elicited but was felt to have been at least 16–20 years prior to testing in one case. Family members remained seronegative (Schweizer *et al.*, 1997). Polymerase chain reaction (PCR) studies found DNA from foamy virus in two human recipients of baboon liver transplants (Allan *et al.*, 1998). The virus was not able to be isolated despite multiple cultures; likewise, serologic studies on the human recipients remained negative after transplantation. Accordingly, the interpretation of the finding of DNA from foamy virus in these human xenografts recipients is unclear; it may represent microchimerism rather than true infection of human cells. Despite the problems with interpretation in these two cases, the risk of infection appeared to be high albeit the proof of clinical relevance remained unknown. Exogenous retroviruses of swine are less well characterized than those of non-human primates. Some pig retroviruses reactivate after exposure to radiation and therefore may be at risk for reactivation under the influence of immunosuppressive drugs (Frazier, 1985). While it may prove to be laborious, it would seem prudent to screen potential swine donors that are considered for xenotransplantation that harbor exogenous retroviruses.

Endogenous retroviruses, normal genetic elements encoded in the chromosome, have been raised as concerns for xenotransplantation in particular because they cannot be removed from source animal populations by current rearing methods. These viruses have long been recognized but only with the renewal of interest in clinical xenotransplantation have more in-depth studies of endogenous retroviruses begun to be conducted. All strains of pigs studied carry porcine endogenous retroviruses (PERVs) within their genome (Patience *et al.*, 1997; Le Tissier *et al.*, 1997; Martin *et al.*, 1998; Wilson *et al.*, 1998; Takeuchi *et al.*, 1998). Likewise, baboon endogenous virus (BaEV) is found in baboon genomes. *In vitro* studies show both PERV and BaEV capable of infecting human cell lines (Patience *et al.*, 1997; Le Tissier *et al.*, 1997; Martin *et al.*, 1998; Wilson *et al.*, 1998; Takeuchi *et al.*, 1998; Huang *et al.*, 1989). A human who received baboon bone marrow for experimental treatment of HIV-1 had transient presence of BaEV detected at day 5 after transplantation but not subsequently (Michaels *et al.*, 2004). The finding was in conjunction with finding baboon mitochondria, again limiting interpretation as to whether this represented chimerism versus true infection. The largest review to date evaluated 160 people who had various pig tissue transplants up to 12 years earlier (Paradis *et al.*, 1999). No patient was found to have detectable viremia. In addition, no persistent PERV infection could be found; 23 patients had evidence of microchimerism up to 8 years after exposure to pig tissues. Likewise, more recent studies looking at other groups of patients exposed to porcine cells over time have not shown infection with PERV (DiNicuolo *et al.*, 2010). Long-term follow-up of eight patients surviving treatment with porcine cell-based Academic Medical Center bioartificial liver found no evidence of PERV DNA in peripheral blood mononuclear cells (PBMCs) nor RNA in plasma or PBMC samples (DiNicuolo *et al.*,

2010). Extensive reviews on the subject likewise failed to find viremia, but caution remains about the potential risk particularly with recombination of PERV-A and PERV –C strains (Denner and Tonjes, 2012; Fishman *et al*., 2012).

Other virus classes have been transmitted after allotransplantation and may likewise cause disease after xenotransplantation. For example, unrecognized acute viremia with adenovirus has caused graft failure after human liver transplantation (Varki *et al*., 1990). Since adenovirus can infect many animal species similar concerns may exist. Alternatively, it is possible that xenotransplantation may cause less of a risk for this type of transmission of acute infectious agents because animals can be reared under much more stringent conditions where surveillance for infections or unwellness could be detected.

HBV and HCV have been transmitted after allotransplantation and bring up another area where xenotransplantation has been suggested as a superior alternative to human grafts. Baboons appeared to be resistant to infection with HBV (Starzl *et al*., 1993, 1994; Michaels *et al*., 1996). Accordingly, livers from baboons were used in an attempt to transplant two patients with end-stage HBV liver disease (Starzl *et al*., 1993, 1994). Subsequent research showed that under experimental conditions HBV could actually infect baboon livers and alternative strategies to xenotransplantation using antiviral agents in combination with hepatitis B immunoglobulin have permitted successful allotransplantation for people with HBV infection. Some hepatitis viruses are not species-specific and could pose a risk for xenotransplantation; related strains of hepatitis E virus have been found to cross species barriers (Meng *et al*., 1998).

Numerous other viruses, some long recognized and others newly recognized, in animal populations can be added to the growing lists of potential xenozoonotic infections. Examples include reoviruses, circoviruses, and paramyxoviruses (Public Health Service, 2001; Philbey *et al*., 1998; Halpin *et al*., 1999). Menangle virus is a paramyxoviral zoonosis infecting pigs and humans in Australia and believed to be harbored by flying foxes (Halpin *et al*., 1999). Coronaviruses in particular can cross species lines with fatal results as found with severe acute respiratory syndrome (SARS) and more recently the Middle East respiratory syndrome (MERS) (Peiris *et al*., 2003; Reusken *et al*., 2013). It is imperative that animal sources are maintained under strict rearing methods and issues of rodent, insect, or other animal infestation considered. For more information, please refer to The Public Health Service guideline on infectious disease in xenotransplantation (Public Health Service, 2001) and other relevant reviews (Denner and Tonjes, 2012; Fishman *et al*., 2012).

In addition to infection of a xenotransplant recipient with an animal virus, consideration must also be given to the possibility of recombination (Chou, 1989; Halliburton *et al*., 1977; Isfort *et al*., 1992). Mixed-strain isolates of human CMV can recombine *in vitro* with passage (Chou, 1989). Mouse studies have demonstrated that infection with two avirulent HSV can lead to recombinations that are lethal (Halliburton *et al*., 1977). *In vitro* integration of reticuloendotheliosis virus (an avian retrovirus) into an avian herpesvirus (Marek disease virus) can lead to lethal recombinant strains (Isfort *et al*., 1992). Consideration should also be given to whether it is possible for avian influenza to gain access to swine tissue within a xenotransplant recipient leading to mixing of influenza within a human host. In addition, as mentioned earlier, consideration for infection of porcine or other xenogeneic tissue with human viruses could lead to graft dysfunction (Millard and Mueller, 2010).

IV. NONVIRAL AGENTS

Nonviral agents may also cause xenogeneic infections if present in tissues being transplanted such as toxoplasma cysts in the cardiac muscle of a transplanted heart, regardless of the donor species. Throughout the world, commercial herds of swine are commonly seropositive for *T. gondii* (Hill and Dubey, 2013). Parasites normally confined to the gastrointestinal tract could be a problem if extra-intestinal infection occurs as is possible for *Entamoeba histolytica* and some schistosoma species. Local epidemiologic considerations will influence the types of parasites considered. Source animal populations should be raised in protected environments, where parasitic infestation is avoided.

Bacterial and fungal diseases, while less likely to be latent, still require consideration. For example, mycobacterium species can infect animals including baboons and swine which may not manifest clinical symptoms until disseminated end-stage disease. Often the source of tuberculosis in animal populations is from human caretakers; accordingly, serial tuberculin skin testing of human caregivers and source animals should be routine. Prions likewise need to be considered when using animal neurogenic cells for diseases such as Parkinson's disease, although prion disease has not been found thus far in swine.

V. DEVELOPMENT OF SPF HERDS

Many concerns for xenozoonoses could be diminished if source animals could be raised under germ-free conditions. Small laboratory animals have been raised in gnotobiotic environments. Pigs have also been raised under these conditions to a lesser extent, but have

problems after several months of age because of their size and waste (stool and urine) production. These environments also preclude the typical colonization of the gastrointestinal tract with normal microbial agents that help with the digestion of food. For this reason, pigs raised in germ-free conditions are less robust and may not be ideal organ sources (Michaels and Simmons, 1994; Chapman, 1995; Public Health Service, 2001; Fishman, 1994). Consideration therefore turned to raising source animals under controlled environments in which specific pathogens have been eliminated and the introduction of outside pathogens is guarded against (Michaels and Simmons, 1994; Chapman, 1995; Public Health Service, 2013; Fishman *et al.*, 2012; Fishman, 1994). Vigilance against the accidental introduction of microbes to an established colony must be strictly maintained from human caretakers or outside animals. In addition, concern remains for the possibility of xenogeneic infection from microbes that are currently not identified and therefore not screened out of the population.

Decisions regarding which microbial agents to be screened out of a source animal population need to be reviewed and periodically updated as new infections information is available. For example, the protocol designed for screening a baboon to be used for a bone marrow xenotransplant to attempt to reconstitute the immune system in a person with AIDS-classified microbes into one of four groups: (1) absolute contraindications, (2) relative contraindications, (3) treatable microbes, or (4) unavoidable microbes (Michaels *et al.*, 2004). Absolute contraindications included microbial agents that were known to be zoonotic and dangerous to humans, even if they were not anticipated to be found in baboons that were born and raised in the United States. For example, SIV, STLV, filoviruses, *T. gondii*, *M. tuberculosis*, and herpes B virus were put in this category, even though SIV and herpes B were not anticipated in baboon populations at all. Relative contraindications consisted of microbes that were hypothesized to be xenozoonotic but were unproven or with uncertain consequence. Examples included baboon herpesviruses and foamy virus. Treatable infections were microbes that could be identified and eradicated prior to bone marrow harvest such as *Babesia* species or gastrointestinal pathogens. The fourth category, 'unavoidable organisms' included BaEV and microbes that exist but were as yet unrecognized and thus clearly of indeterminate risk (Michaels *et al.*, 2004). The categories were developed with the most up-to-date information available but also with the recognition that future protocols might move some of the infectious agents into different classes. The same is true for screening lists for swine and other species considered for source animals. In addition to developing protocols for which organisms should be evaluated, it is important to determine which testing method will be used and to be prepared to change these methods as more sophisticated techniques are developed (Fishman *et al.*, 2012). As noted previously, not all testing methods are equivalent. For example, a serologic survey of baboons raised in the United States demonstrated great variability in identifying baboons with evidence of *H. papio*, the EBV analog (Michaels *et al.*, 1994). This highlights the need to develop more sensitive techniques specific to the agent being evaluated. One approach to increase the sensitivity of screening was used when screening baboons for liver transplantation; paired sera samples from potential source animals were sent to two laboratories using different techniques to look for a wide variety of viruses; any positive finding was classified as a true positive (Michaels *et al.*, 1994). Also recognizing that serologic surveys have the potential to miss an immunologic response to a recently encountered agent, selected animals that tested negative initially were quarantined and retested over time for these same potential pathogens.

Swine can be reared efficiently in controlled environments, which makes screening somewhat easier. However, prospective considerations for the types of screening are still necessary (Public Health Service, 2011; Fishman *et al.*, 2012; Fishman, 1994; Ye *et al.*, 1994). One study evaluated 10 newborn piglets that were reared in a brucellosis, pseudorabies virus, atrophic rhinitis, and *Mycoplasma hyponeumonia* (Ye *et al.*, 1994). The investigators cultured the skin, urine, feces and nasal swabs for bacteria and examined the tissues for fungi and parasites. Further testing included bacterial blood culture commercial serologic tests for antibody against human CMV, HBV, HCV, HIV, *T. pallidum*, and *T. gondii*. Tests were performed serially and at necropsy. No pathogens were identified which the investigators considered as a risk for xenotransplantation. However, 2 of the 10 pigs had positive enzyme-linked immunosorbent assay (ELISA) tests for HIV at one point in time. Further investigation revealed them to be negative, but this again emphasizes limitations that can exist with screening techniques.

VI. POTENTIAL BENEFITS OF XENOTRANSPLANTATION AND OTHER INFECTIOUS DISEASE ISSUES

It is possible that xenotransplantation may decrease the risk of some infections after transplantation. For example, in the 1980s allotransplantation was not an ideal treatment for humans with HBV or HIV because the virus would infect the new organ or hematopoietic cells. The resistance of baboon livers to HBV was the rationale for xenotransplantation in two patients with end-stage liver disease from HBV (Starzl *et al.*, 1993, 1994). Likewise, the rationale of attempting to reconstitute the immune system of a patient with HIV through xenotransplantation

was again because of the natural resistance of the baboon to HIV-1 (Michaels *et al.*, 2004). Newer strategies for treating both viruses emerged, and accordingly the risk benefit weighed against the use of xenotransplantation for these particular diseases but conceptually an animal organ may still have this type of benefit. As noted previously, xenotransplantation may avoid many donor-transmitted infections by permitting source animals to be reared in controlled, SPF environments and surveyed against acute infections. Performing transplants as elective surgery rather than as emergency procedures which is often required with allotransplantation would also decrease postoperative infectious complications.

VII. ISSUES AFTER XENOTRANSPLANTATION

SPF-controlled and well-regulated environments are critical to decrease the risk of xenozoonoses, but will not eliminate it completely. For this reason, it is important for any recipient of xenogeneic tissue to undergo counseling about the potential risks and surveillance for new infections after xenotransplantation. In this fashion, the true epidemiology and risks of xenotransplant infections will be recognized. Serial samples from the recipient and transplanted tissues should be collected for cultures and/or assays to look for agents that were known or suspected to be in the source animal, such as endogenous viruses. These recommendations were added into the guidelines recommended by the United States Public Health Services (PHS) and World Health Organization (WHO) (Public Health Service, 2001; Fishman *et al.*, 2012). However, as noted, current techniques for screening may ultimately prove inadequate. Accordingly, archiving samples for future studies are important and should be maintained for a minimum of 50 years (Public Health Service, 2001; FDA, 2003). Shared or centralized registries and repositories for archived specimens may help with evaluating potential infectious agents; however, at this time they are not available. Initially, the Department of Health and Human Services formed a Secretary's Advisory Committee on Xenotransplantation as well as the FDA Biological Response Modifiers Advisory Committee (BRMAC) to consider the complexities of xenotransplantation and to help with ongoing review of these procedures. However, xenogeneic activity is now directly under the auspices of the FDA Cell, Tissue and Gene Therapies Advisory Committee (CTGTAC), which coordinate and participate with global groups such as the WHO (FDA, 2003).

All biologic agents have an inherent risk for transmitting infections and our ability to recognize and prevent these infections is continuously growing. Xenotransplantation has the potential to offer life-saving tissues and grafts to a number of people who currently die because of the absence of available human donors; as the field grows, it is imperative that new techniques be developed to help identify and prevent novel infections.

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