Infection in Xenotransplantation: Organ-Source Health and Patient Safety

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Abbreviations

PERV Porcine endogenous retrovirus

Introduction: Infection in Transplantation

Infection and cancer are major complications of long-term immunosuppressive therapy used to prevent graft rejection. Infection is derived from environmental exposures in the hospital and the community, from organisms present, often as colonizers or in latent form, in the organ recipient and from organisms carried with the transplanted organ [1–5]. In xenotransplantation, data regarding the microbiology of normal and genetically modified swine are limited. Approaches to the mitigation of the infectious risks of xenotransplantation are based on extrapolation from experience with infection following allotransplantation and on preclinical data developed in studies of immunosuppressed swine and primate xenograft recipients (Table 17.1).

Based on these data, creative strategies have been developed to minimize xenogeneic infectious exposures via screening of source animals and exclusion of potential pathogens (or animals) during animal husbandry. Routine monitoring of xenograft recipients for infection due to both known and unknown pathogens will be complemented, if infectious syndromes emerge, by standard paradigms for
management of immunocompromised hosts with infection. As for allotransplantation, there will always remain some irreducible infectious risk associated with transplantation of viable xenograft tissues in graft recipients with organ dysfunction and other medical problems and who are undergoing complex surgical procedures and intensive immunosuppressive therapy.

The risk of infection in transplantation is determined by the semiquantitative relationship between two factors, the “epidemiologic exposures” and a conceptual measure of an individual’s susceptibility to infection termed the “net state of immunosuppression.” The net state of immunosuppression is largely a function of the intensity of immunosuppressive therapy but also includes metabolic derangements, infection with immunomodulating herpes and other viruses, and technical complications (e.g., devitalized tissues, undrained fluid collections). A decreased risk of infection (e.g., due to donor screening or recipient prophylaxis) increases the tolerability of immunosuppression. The risk factors for allotransplantation have been reviewed elsewhere [1, 2]. The unique features of xenotransplantation result from the microbiology of the nonhuman organ donor and the possibility that greater-than-usual immunosuppressive therapy may be required to prevent graft rejection [5–8].

**Table 17.1** Categories of potential opportunistic infections resulting from clinical xenotransplantation

| Common pathogens: community or nosocomially acquired organisms causing infection (e.g., wound infection, pneumonia), specific diagnostic tests generally available, effective therapies available |
|---|
| Opportunistic infections of the immunocompromised host: well-characterized clinical syndromes in human allograft recipients (e.g., cytomegalovirus infection), specific diagnostic tests generally available, effective therapies available |
| “Traditional zoonosis”: well-characterized clinical syndromes in humans (e.g., *T. gondii*), specific diagnostic tests generally available, effective therapies available |
| “Species specific”: incapable of causing infection outside the xenograft (e.g., porcine CMV), some tests available, few standardized tests available for human use |
| “Potential pathogens”: organisms of broad “host range” which may spread beyond the xenograft (e.g., adenovirus), few specific tests available, some effective therapies available |
| “Unknown” pathogens: unknown clinical and microbiological characteristics in vivo in humans (e.g., porcine endogenous retrovirus, PERV), some tests available, some therapies available |
| New virulence characteristics within the host (e.g., xenotropic viruses) |
| Not known to be present or pathogenic (e.g., protozoa or retroviruses) |
| Viral recombinants |

*Assays must be validated for use in swine and in human samples*

**Xenosis: Which Pathogens?**

The term “xenosis” (also “direct zoonosis” or “xenozoonosis”) reflects the unique epidemiology of infection due to organisms from a nonhuman source species transmitted with xenogeneic grafts [6, 8–10]. It must be emphasized that any organism can potentially cause infection in immunocompromised hosts, so discussion must
focus on what are considered the likely pathogens based on experience with allotransplantation (Table 17.1). The microbiological behavior of animal-derived pathogens in the immunosuppressed human host cannot be predicted, and the clinical manifestations of infection are altered by immunosuppression. Various factors may increase the risk of infection in xenotransplantation:

(i) Potential pathogens may be of microbial species previously unappreciated (porcine endogenous retroviruses, PERV) or unexamined (e.g., polyomaviruses) in the source species [11–15].

(ii) Novel clinical syndromes may result from infection with animal-derived pathogens.

(iii) Clinical laboratory assays for organisms from nonhuman species may not be available for use in donor screening or in clinical diagnosis.

(iv) Donor-derived organisms may be nonpathogenic in the native species but cause disease in the new host (“xenotropic organisms”) or acquire new characteristics (genetic recombination or mutation) [16–21]. Virulence may increase with passage in a new host (evolutionary adaptation) while diminishing over time in the native host.

(v) As in allotransplantation, incompatibility of transplantation antigens (i.e., MHC antigens) between species may reduce the efficacy of the host’s immune response to infection within the xenograft.

As for allotransplantation, keys to the management of infection derived from swine include:

(i) Identification of “likely” pathogens based on experience with related organisms in allotransplant recipients (Table 17.1). In the absence of clinical trials, such predictions are merely educated guesses.

(ii) Development of sensitive and specific microbiological assays for use in breeding, donor and organ screening, and diagnosis. Ideally, this would include serological tests and/or measures of T-lymphocyte immunity (e.g., pathogen-specific interferon-gamma release assays) to identify prior exposures and latent infections. In addition, culture systems, microscopic analyses (for parasites), and quantitative molecular assays for use in clinical diagnosis are needed. These must be validated for use with samples from swine and from human xenograft recipients as assays may perform differently in human and porcine sera. Thus far, serological testing for most animal-derived organisms in humans is generally unavailable or unreliable. Serological tests may also be falsely negative in the immunocompromised host. Such assays are available in small numbers of commercial or veterinary programs.

(iii) Identification of therapies appropriate for each pathogen.

With these tools, an “exclusion list” of organisms thought to pose an unaccept-able risk to xenograft recipients has been developed as a basis for testing in breeding colonies (“Designated Pathogen-Free Colonies” (Table 17.2) [9, 13, 22–24]). While
barrier facilities to prevent infection of breeding colonies are essential, it seems likely that it does not matter how such exclusion is achieved if the designated organisms are demonstrably absent from the transplanted organ. Such lists must be dynamic – subject to revision based on experimental and clinical experience and the availability of new therapies.

Source Animal Selection and Exclusion of Likely Pathogens

The need for herd isolation and continuous surveillance of source animals requires meticulous breeding records (including details of nuclear transfer and animal movements) and archiving of specimens (cells and sera) for subsequent use in epidemiological investigations. Microbiological assessments in breeding colonies will be needed for sentinel animals and from the specific animals selected for organ procurement. Swine for xenotransplantation may be bred in “biosecure facilities” to prevent introduction of pig or human pathogens and isolated from other animals, including rodents, insects, and birds, often with care providers gowned and gloved.

We have developed two lists of organisms for consideration in breeding for xenotransplantation. Pig health is assured by standard veterinary practice including routine vaccinations with microbially restricted and mammalian protein-free diets, filtered water, and special housing and avoidance of unnecessary antibiotics (Table 17.3). With the availability of genetic modification of swine (e.g., CRISPR-Cas9) targeting graft rejection, metabolic incompatibilities, or to eliminate endogenous retroviruses, transgenic methods with nuclear transfer are performed in sterile environments, with subsequent embryo transfer to surrogate gilts [25–31].

| **Viruses** | **Bacteria** | **Parasites** |
|---|---|---|
| Porcine endogenous retrovirus (PERV) A, B, C, AC | *Porcine lymphotrophic herpesvirus (PLHV)* | *Mycobacteria* spp. | *Porcine adenovirus* | *Porcine teschovirus* | *Shigella* |
| Encephalomyocarditis virus | Rabies virus | *Pathogenic E. coli* | *Porcine cytomegalovirus* | *Swine influenza virus* | *Yersinia* |
| Hepatitis E virus | West Nile virus | *Campylobacter* | *Porcine hemagglutinating encephalomyelitis* | *Encephalomyocarditis virus* | *Leptospira* spp. |
| *Porcine lymphotrophic herpesvirus (PLHV)* | *Rabies virus* | *Salmonella (choleraesuis, typhimurium)* | *Hepatitis E virus* | *Swine influenza virus* | *Listeria* spp. |
| *Porcine teschovirus* | *West Nile virus* | *Toxoplasma gondii* | *Porcine hemagglutinating encephalomyelitis* | *Rabies virus* | *Echinococcus* spp. |
| *Swine influenza virus* | *Encephalomyocarditis virus* | *Cryptosporidium parvum* | *Rabies virus* | *Listeria* spp. | *Trichinella spiralis* |
| *Porcine hemagglutinating encephalomyelitis* | *SARS-Cov-1 and 2* | *Strongyloides* | *Trypanosoma species* | *Listeria* spp. | *Microsporidium* |

Adapted from [8]
Based on experience with infections in immunosuppressed human allotransplant recipients and with pig-to-primate xenotransplantation, a second “Designated Pathogen-Free Exclusion List” was developed (Table 17.2). Thus far, infections due to pig-derived pathogens have not been identified in immunosuppressed humans, except for hepatitis E virus (HEV). Regulatory guidance documents exist for clinical trials [10, 32–35]. In practice, these documents require source animal screening to assure animal health and the absence, to the degree possible, of possible pig-derived human pathogens.

### Table 17.3 Exclusion list: organisms important to swine health status

| Viruses                          |
|----------------------------------|
| Parvovirus                       |
| Porcine circovirus               |
| Porcine delta coronavirus         |
| Porcine diarrhea virus           |
| Porcine reproductive and respiratory virus |
| Porcine respiratory coronavirus  |
| Porcine sapelovirus 1            |
| Pseudorabies or Aujeszky’s disease |
| Transmissible gastroenteritis virus |
| Bacteria                         |
| Brucella suis                    |
| Leptospira spp.                  |
| Mycoplasma hyopneumoniae         |
| Salmonella spp.                  |
| Parasites                        |
| Ascaris suum                     |
| Cryptosporidia                   |
| Strongyloides ransomi            |

Adapted from [8]

### Safety in Clinical Trials of Xenotransplantation

#### Routine Monitoring for Xenogeneic Infection

In immunosuppressed organ recipients, the risks for infection and malignancy are lifelong. Standard pretransplant screening in advance of immunosuppressive therapy is required (Table 17.4). While most donor-derived infections are identified early in the posttransplant course, some infections occur later, often due to immune perturbation by intercurrent viral infection (e.g., cytomegalovirus) or augmented immunosuppression for graft rejection [3, 4, 36–38]. Proof that the source of such infections is swine-derived vs. environmental may be impossible. Based on the technologies applied (e.g., molecular testing, next-generation sequencing), routine samples from recipients might be tested to assure the absence of potential pathogens (e.g., porcine endogenous retrovirus, porcine cytomegalovirus) (Tables 17.5 and 17.6). Similar screens might be applied at times of symptomatic infection or of
Table 17.4  Pretransplant microbiological screening of human xenograft recipients

| Name                                               | Testing method(s) |
|----------------------------------------------------|-------------------|
| Human immunodeficiency virus, type 1 (HIV-1)       | ELISA             |
| Human immunodeficiency virus, type 2 (HIV-2)       | ELISA             |
| Hepatitis B virus                                  | Serology          |
| Hepatitis C virus                                  | Serology          |
| *Treponema pallidum*                              | Serology          |
| Human cytomegalovirus (CMV)                        | Serology          |
| Human herpes simplex virus                         | Serology          |
| Human varicella zoster virus                       | Serology          |
| *Toxoplasma gondii*                                | Serology          |
| *Mycobacterium tuberculosis*                       | ELISA (T-spot)    |

*aVaccine status up to date for hepatitis B; hepatitis A; influenza virus; Pneumovax/PCV13; tetanus (Tdap); MMR (measles, mumps, and rubella); varicella zoster virus; if required: meningococcal (including type B), H. influenzae; human papillomavirus*

Table 17.5  Deployment of microbiological assays in xenotransplantation

| Assay type                                                      | Screening source animals | Xenograft recipient monitoring | Xenograft recipients – symptomatic infection or increased riska | Healthy contacts of recipient |
|----------------------------------------------------------------|--------------------------|-------------------------------|---------------------------------------------------------------|-------------------------------|
| Cultures (active infection)                                     | X                        |                               | X                                                             |                               |
| Serology (past exposures)                                       | X                        | X                             | +/-                                                          | X                             |
| Molecular assay or antigen detection (active infection)        | X                        |                               |                                                                |                               |
| Next-generation sequencing (active infection)                  | X                        | X                             |                                                              |                               |

*aIncreased risk may be associated with treatment of graft rejection or intercurrent viral infection*

Table 17.6  Recipient testing (post-xenotransplantation routine)

| Virus name – noncommercial testing | Testing method |
|------------------------------------|----------------|
| Porcine endogenous retrovirus (PERV) A, B, C, AC | Qualitative and quantitative (QNAT) nucleic acid testing (NAT); antibody-based tests (serology, ELISA, Western blot) |
| Porcine lymphotrophic herpesvirus type 2 (PLHV-2) | QNAT         |
| Porcine cytomegalovirus (PCMV)       | NAT; antibody-based tests |
| Human cytomegalovirus (HCMV) – per protocol | QNAT |
| Human Epstein-Barr virus (EBV) – per protocol | QNAT |

Adapted from [8]
increased risk (e.g., following treatment of graft rejection). In addition to recipient samples, social or sexual contacts of recipients and source-animal handlers may be considered for inclusion in any monitoring scheme. For this reason, sera and cells from these groups must be archived for future studies.

Recipients of xenografts should have blood samples (sera and cells) obtained and stored at regular intervals. A possible scheme might include serum and leukocyte samples (Table 17.6):

(i) Pretransplant
(ii) Weekly for 1 month postoperatively
(iii) Monthly for 6 months postoperatively
(iv) Quarterly for the first year
(v) Annually for 5 years thereafter

Following periods of fever or of clinical infection (see below), monitoring would be increased to weekly for 1–2 months and then revert to the previous level of surveillance. Samples could be stored on relatives, intimate contacts, and animal handlers every 6 months, with more frequent monitoring (monthly) if the animals or recipients developed signs of infection or were determined to be infected with a xenograft-derived pathogen.

Samples will be used for (i) archiving for future epidemiologic studies (in appropriate storage media for RNA, DNA, cell, and antibody preservation); (ii) NAT testing for PERV (A, B, C, AC), PLHV, and PCMV (if present in donor) and for common human viruses; (iii) cocultivation of peripheral blood leukocytes with permissive human and porcine cell lines for viral detection (including PERV); and (iv) evaluation for any fevers or infectious syndrome per institutional protocols (Tables 17.5 and 17.6).

With periods of fever or of clinical infection, monitoring could increase (e.g., to weekly for 1–2 months and then revert to the previous level of surveillance) depending on the diagnosis obtained. Samples should be stored from social contacts and animal handlers (e.g., every 6 months), with more frequent monitoring (monthly) if the animals or recipients develop signs of infection. Both serologic and molecular assays must be validated for human blood samples.

**Management of Xenograft Recipients with Signs of Infection**

Organ transplant recipients frequently manifest signs of infection in the form of fever (often without clear source); unexplained leukocytosis; graft dysfunction; respiratory, gastrointestinal, or urinary tract symptoms; sepsis; or abnormal metabolic testing (e.g., hepatitis). Graft rejection and malignancy may present similarly. Most often, these signs and symptoms reflect community-acquired infections or reactivation of latent infections. The risk of xenograft-derived infection requires approaches like those of allograft recipients:
(i) Full microbiological evaluation prior to the initiation of antimicrobial therapy (blood and urine cultures, sputum cultures)
(ii) Radiologic studies and invasive diagnostic testing (needle or surgical biopsies) as appropriate
(iii) Early empiric antimicrobial therapy directed at the most likely pathogens
(iv) Hospital admission with isolation and infectious precautions until further data become available
(v) Universal precautions for all blood samples
(vi) Special testing based on data from the source animals with consideration of both pig-specific pathogen testing and nondirected sequencing of serum samples

The First Recipients

Ideally, initial xenograft recipients would undergo transplantation in the absence of immunosuppression. These might be recipients of porcine skin grafts used for transient wound or burn coverage until sloughed. Such recipients could be assessed for xenogeneic infection locally (at the site of application) and systemically. Significant advances in preclinical studies have demonstrated good xenograft survival using clinically acceptable approaches to immunosuppression. Subsequent recipients requiring immunosuppressive therapy should be free of known infections and not be colonized with antimicrobial-resistant organisms. Infectious risk to the xenograft recipient might be increased by preexisting immunodeficiency states in candidates for xenotransplantation and may mitigate against using xenografts in prior allograft recipients or with underlying immunodeficiencies. Protocols for graft tolerance induction (e.g., stem cell plus organ grafts from the same donor) may avoid the intensive immunosuppression required to maintain graft function in primates but assume systemic spread of pig cells in the recipient with the associated risks of infection and graft-vs.-host disease.

In xenotransplantation, in the absence of human studies, the absolute risk for infections remains unknown. Approaches to production and modification of source animals and surveillance in recipients will require adjustment as clinical data emerge. New microbiological assays will be required to screen swine for potential human pathogens and for the diagnosis of pig-specific pathogens in humans. The application of next-generation sequencing technologies to xenograft recipient samples may provide valuable data and another layer of clinical safety. Infections occurring in the xenograft recipient will require early diagnosis and therapy. However, it is unlikely that such opportunistic infections will pose a significant risk to immunologically normal individuals. The recognition that novel organisms may infect xenograft recipients should generate improvements in technologies for the screening of source animals and surveillance of recipients.

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References

1. Fishman JA. Infection in solid-organ transplant recipients. N Engl J Med. 2007;357:2601–14.
2. Fishman JA. Infection in organ transplantation. Am J Transplant. 2017;17:856–79.
3. Fishman JA, Greenwald MA, Grossi PA. Transmission of infection with human allografts: essential considerations in donor screening. Clin Infect Dis. 2012;55:720–7.
4. Fishman JA, Grossi PA. Donor-derived infection—the challenge for transplant safety. Nat Rev Nephrol. 2014;10:663–72.
5. Fishman JA, Patience C. Xenotransplantation: infectious risk revisited. Am J Transplant. 2004;4:1383–90.
6. Fishman JA. Infection in xenotransplantation. BMJ. 2000;321:717–8.
7. Fishman JA, Scobie L, Takeuchi Y. Xenotransplantation-associated infectious risk: a WHO consultation. Xenotransplantation. 2012;19:72–81.
8. Fishman JA. Infectious disease risks in xenotransplantation. Am J Transplant. 2018;18:1857–64.
9. Fishman JA. Infection in xenotransplantation. J Thorac Cardiovasc Surg. 2001;16:363–73.
10. WHO. Second WHO global consultation on regulatory requirements for xenotransplantation clinical trials. 2001.
11. Peretti A, FitzGerald PC, Bliskovsky V, Buck CB, Pastrana DV. Hamburger polyomaviruses. J Gen Virol. 2015;96:833–9. https://www.who.int/transplantation/xeno/report2nd_global_consultation_xtx.pdf?ua=1.
12. Akiyoshi DE, Denaro M, Zhu H, et al. Identification of a full-length cDNA for an endogenous retrovirus of miniature swine. J Virol. 1998;72:4503–7.
13. Fishman JA, Patience C. Xenotransplantation: infectious risk revisited. Am J Transplant. 2004;4:1383–90.
14. Hause BM, Smith C, Bishop B, Stewart C, Simonson R. Complete genome sequence of a porcine polyomavirus from nasal swabs of pigs with respiratory disease. Genome Announc. 2018;6:e00344-18.
15. Denner J. The porcine virome and xenotransplantation. J Virol. 2017;14:171.
16. Coffin JM, Stoye JP, Frankel WN. Genetics of endogenous murine leukemia viruses. Ann N Y Acad Sci. 1989;567:39–49.
17. Frankel WN, Stoye JP, Taylor BA, Coffin JM. Genetic analysis of endogenous xenotropic murine leukemia viruses: association with two common mouse mutations and the viral restriction locus Fv-1. J Virol. 1989;63:1763–74.
18. Rando RF, Srinivasan A, Feingold J, Gonczol E, Plotkin S. Characterization of multiple molecular interactions between human cytomegalovirus (HCMV) and human immunodeficiency virus type 1 (HIV-1). Virology. 1990;176:87–97.
19. Isfort R, Jones D, Kost R, Witter R, Kung HJ. Retrovirus insertion into herpesvirus in vitro and in vivo. Proc Natl Acad Sci U S A. 1992;89:991–5.
20. Javier RT, Sedarati F, Stevens JG. Two avirulent herpes simplex viruses generate lethal recombinants in vivo. Science. 1986;234:746–8.
21. Katz RA, Skalka AM. Generation of diversity in retroviruses. Annu Rev Genet. 1990;24:409–45.
22. Fishman JA. Xenosis and xenotransplantation: addressing the infectious risks posed by an emerging technology. Kidney Int Supple. 1997;58:S41–5.
23. Fishman JA. Infection and xenotransplantation. Developing strategies to minimize risk. Ann N Y Acad Sci. 1998;862:52–66.
24. Fishman JA. The risk of infection in xenotransplantation. Introduction. Ann N Y Acad Sci. 1998;862:45–51.
25. Klymiuk N, Aigner B, Brem G, Wolf E. Genetic modification of pigs as organ donors for xenotransplantation. Mol Reprod Dev. 2010;77:209–21.
26. Hryhorowicz M, Zeyland J, Słomski R, Lipiński D. Genetically modified pigs as organ donors for xenotransplantation. Mol Biotechnol. 2017;59:435–44.
27. Yang L, Guell M, Niu D, et al. Genome-wide inactivation of porcine endogenous retroviruses (PERVs). Science. 2015;350:1101–4.
28. Sachs DH, Galli C. Genetic manipulation in pigs. Curr Opin Organ Transplant. 2009;14:148–53.
29. Phelps CJ, Koike C, Vaught TD, et al. Production of alpha 1,3-galactosyltransferase-deficient pigs. Science. 2003;299:411–4.
30. Niu D, Wei HJ, Lin L, et al. Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. Science. 2017;357:1303–7.
31. Schaefer KA, Wu WH, Colgan DF, Tsang SH, Bassuk AG, Mahajan VB. Unexpected mutations after CRISPR-Cas9 editing in vivo. Nat Methods. 2017;14:547–8.
32. Hering BJ, Cooper DK, Cozzi E, et al. The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes – executive summary. Xenotransplantation. 2009;16:196–202.
33. Hering BJ, Cozzi E, Spizzo T, et al. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes--executive summary. Xenotransplantation. 2016;23:3–13.
34. Food and Drug Administration C, U.S. DHHS. Source animal, product, preclinical, and clinical issues concerning the use of xenotransplantation products in humans. Guidance for Industry, 2016.
35. PHS. PHS guideline on infectious disease issues in xenotransplantation. Rockville: Food and Drug Administration, Center for Biologics Evaluation and Research; 2001. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.
36. Fishman JA. New technologies for infectious screening of organ donors. Transplant Proc. 2011;43:2443–5.
37. Grossi PA, Fishman JA. Donor-derived infections in solid organ transplant recipients. Am J Transplant. 2009;9(Suppl 4):S19–26.
38. Michaels MG, McMichael JP, Brasky K, et al. Screening donors for xenotransplantation. The potential for xenozoonoses. Transplantation. 1994;57:1462–5.