Human Xenograft Transplantation in Animal Research: Risk Assessment and Hazard Control for Animal Care Workers

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Introduction

Much progress in the field of cancer research can be attributed to the use of well-established animal models. The ability to transplant human cells and tissue, such as tumors and other malignant cells, into immunocompromised research animals has proven to be of great importance. Studies to identify new antineoplastic agents, determine their effectiveness in suppressing tumor growth, and to measure their toxicity, all require the use of this animal model. This system is often the first stage leading to human clinical trials that result in the possible approval of diagnostic procedures, new drugs, or other treatments. The process of implanting human cells or tissues into research animals, by injection or engraftment, is also referred to as human xenograft transplantation. A host animal is usually genetically or chemically altered to be incapable of rejecting the foreign tissue or cells by using its immunological defense mechanisms. This permits the xenograft to continue growing within the host animal. Immunocompromised rodents, such as those of "nude" or "SCID" varieties, act as effective hosts to the human cells or tissues.

Hazard Assessment

Cells and tissues derived from human sources have the potential for carrying and disseminating infectious agents. This is an elemental premise of the Occupational Safety and Health Administration's (OSHA) Bloodborne Pathogen Standard (1). The standard requires that institutional policies and work practices be present that recognize the risks of, and provide protection from, occupational exposure to bloodborne pathogens and other potentially infectious materials (OPIM). Human cell lines purchased from commercial vendors or repositories, such as the American Type Culture Collection (ATCC), will usually be accompanied by a warning that the cells may not have been tested for the presence of human pathogens, or that testing was limited in scope and may have excluded identification of other potentially infectious agents. Similar caution must be exercised when receiving human cells and tumors from collaborating laboratories. Further passage of these cell lines does not ensure the removal of infectious agents.

Biohazard risk assessments are necessary to determine if there exists a probability of harm, injury, or disease as a result of laboratory practices or procedures. In the case of human xenografts, it is necessary to determine that the source tissue or cells are free of bloodborne pathogens, such as hepatitis viruses, human immunodeficiency virus (HIV), and other viruses or microbes identified as bloodborne pathogens. This determination should be made by a biological safety professional or other qualified scientist with experience to review potential contamination and exposure risk. Testing may include antigenic screening for viral or microbial markers, co-cultivation with various indicator cells that allow contaminants to grow, or using molecular technology, such as the polymerase chain reaction (PCR) or nucleic acid hybridization, to identify viruses that lie latent within the host cell DNA. Such testing is often expensive and may not identify all of the pathogens of concern. Therefore, the risk assessment may conclude that the xenograft material, as well as the implanted research animal host, is considered a potential biohazard.

The presence of infectious agents found in human tissue has been well documented. These agents can include human immunodeficiency virus, the hepatitis viruses, cytomegalovirus (CMV), Epstein-Barr virus (EBV), the Herpes virus family, human T-cell lymphotrophic virus (HTLV), papilloma virus, mycoplasma species, C-J prion, Mycobacterium species, and opportunistic bacteria, fungi, and intracellular parasites. In some cases, this tissue can be obtained from donors who had silent chronic infections. Retroviruses and prions may not produce clinically recognizable disease until years after they enter the host. These are some examples of specific human tissue capable of carrying infectious agents:

- C-J prion in corneal transplants
- Prion-mediated disease and neurotropic viruses in neural tissue xenografts
- Group B streptococcus in human macrophages
- CMV in human neuroblastoma cell lines
- HTLV-1 in choriocarcinoma cell lines
- Simian virus (SV-40 T-antigen) in human brain tumors and tumor cell line
- EBV in human blood and lymphoid tissue
- HIV in human mammary cells and kidneys
- Hepatitis C virus in human heart, liver, and lung
- Human papilloma virus in pharyngo-laryngeal neoplasms, squamous cell carcinomas, and cervical cancer tissue\

Although associated with exposure to infected mice, lymphocytic choriomeningitis (LCM) virus may be acquired by tumors passaged in LCM-infected mice and subsequently transplanted into other rodents.

Human infectious agents have been identified as capable of survival in research animals. Some examples are:

- Group B streptococci in mouse macrophages
- CMV in rat anterior chamber
- Coxsackie virus in mouse heart
- Respiratory syncytial virus in mouse pulmonary system
- Human papilloma virus in mouse
- HIV in modified SCID mouse; SCID mouse with human neural tissue graft
- Rabbit following human T-cell injection
- Mycobacterium avium complex (MAC) in SCID mouse; in Sprague-Dawley rats following treatment with cyclosporine
- Hepatitis C virus in modified SCID mouse
- Chlamydia in SCID mouse
- EBV in SCID mouse

When the risk assessment identifies the presence of a bloodborne pathogen or OPIM, the level of hazard control needs to be established. The CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (2) guidelines provide procedure and containment recommendations based upon the pathogenicity of the biohazardous agent, its possible routes of transmission, the stability of the agent in the environment, its infectious dose, its concentration, the origin of the material, and other factors. These recommendations appear as "Biosafety Levels (BSL)" 1 through 4. As the risk factors increase, so do the Biosafety Levels. Animal research procedures and housing can similarly be assigned to "Animal Biosafety Levels (ABSL)" 1 through 4. When a hazard assessment is unable to identify or exclude the presence of an infectious agent in the xenograft material, Animal Biosafety Level 2 would be the minimum recommended practices. If xenograft materials are tested to be free of all applicable bloodborne pathogens, the results must be documented and made available to all staff participating in the research project.

Hazard Control

Once the human xenograft and its animal host have been assessed as a biohazard and assigned an Animal Biosafety Level, policy and procedure must be drafted to institute procedural controls, select appropriate personal protective equipment and engineering controls, manage waste products, and plan for accident and emergency response. Special procedures may be indicated depending upon the size of the research animal host. These procedures will often accompany the approval of the animal research protocol by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee. Biohazard signs should be posted on animal housing room doors to provide a primary warning, identification of the biohazard (human bloodborne pathogen), and emergency contact information. Personnel that handle these animals and their wastes are advised to follow practices that provide barriers between the biohazards and contact with skin, mucous membranes, or eyes, or accidental ingestion, inhalation, and inoculation. These barriers could be in the form of protective gowns or lab coats, gloves, and safety glasses, goggles, or face shields. Engineering controls may be available such as biological safety cabinets, filter topped cages, or even sharps with safety devices that help prevent accidental needle sticks. Extra care must be taken when handling sharps, such as needles, scalpels, suture needles, and even broken glass, all of which could easily puncture skin and expose an animal handler to potential biohazards. Hand washing or sanitizing with alcohol-based foams upon glove removal is an essential procedure. Eating, drinking, or performing personal hygiene tasks in areas where animal xenograft experiments are in progress should be prohibited. The CDC/NIH biosafety guidelines, and other references (3), include additional standard and special practices, safety equipment to provide primary barriers, and facility design to provide secondary barriers. All used syringes, labware, bedding wastes, and xenograft containing animal carcasses from experiments must be collected as a biohazard waste, usually in used sharps collection containers or autoclavable biohazard bags. These are subsequently submitted for steam sterilization, incineration, or other disinfection method in accordance with regulatory authorities in your area. All work surfaces and equipment in areas where the animals, cells, or bedding has been handled, must be routinely disinfected. This is especially important following a spill or splash of contaminated material. Topical disinfectants should be chosen with specific activity against bloodborne pathogens. A freshly prepared 10 percent household bleach solution, 70 percent ethyl alcohol, or other commercially available disinfectants are good choices. However, they too may be hazardous (ie: flammable, irritant) or corrosive to certain materials (metals), so their careful use must be assessed prior to their selection. Agents such as formalin and glutaraldehyde should not be chosen for routine disinfection practices due to their toxicity and potential for causing acute irritation.

Summary

The use of human xenograft transplants in research animals continues to be a powerful tool for studying cancer biology, imaging technologies, developing new anti-cancer therapies, and determining the pharmacological and toxicological effects of these treatments. It is important to recognize that human cells and tissues have the potential for carrying bloodborne pathogens or other potentially infectious materials. A risk assessment will determine the procedures and special equipment that are necessary to protect workers from accidental exposure to these biohazards. Effective use of personal protective equipment and engineering controls will reduce the risk of biohazard exposure. ■

References:

1. 29 Code of Federal Regulations, Part 1910.1030 "Bloodborne Pathogens" standard.
2. "Biosafety in Microbiological and Biomedical Laboratories", CDC/NIH 4th Edition.
3. "Biosafety in the Laboratory: Prudent Practices for the Handling and Disposal of Infectious Materials"; National Academy Press.