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RETROVIRAL TRANSMISSION IN CONNECTIVE TISSUE ALLOTRANSPLANTATION: AN EXPERIMENTAL STUDY

By

Jean Ann Nemzek

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ABSTRACT

RETROVIRAL TRANSMISSION IN CONNECTIVE TISSUE ALLOTRANSPLANTATION: AN EXPERIMENTAL STUDY

By

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Transmission of retrovirus through connective tissue allotransplantation was studied in an animal model. Feline leukemia virus (FeLV), a retrovirus similar to Human Immunodeficiency Virus (HIV), was used to infect specific pathogen-free (SPF) cats used as tissue donors. Fresh allografts of meniscus (n=4), patellar tendon (n=4), or patellar tendon-bone (n=4) grafts were transplanted from infected donors into the stifles of 12 SPF cats. For positive controls, fresh cancellous bone was transplanted into the proximal tibias of four additional SPF cats. Additional grafts from infected donors (menisci n=4, patellar tendon n=4, patellar tendon-bone n=4) were stored at -80°C for 10 weeks and transplanted into the stifles of 12 other SPF cats. Plasma obtained weekly from all cats was tested for viral antigen and virus-associated antibody titers.

All fresh and fresh-frozen connective tissue allografts transmitted retrovirus as evidenced by the presence of viral antigen and/or rising antibody titers.

This work is dedicated to

Dr. Wade O. Brinker and Dr. Steven P. Arnoczky

for believing in the things that are truly important.

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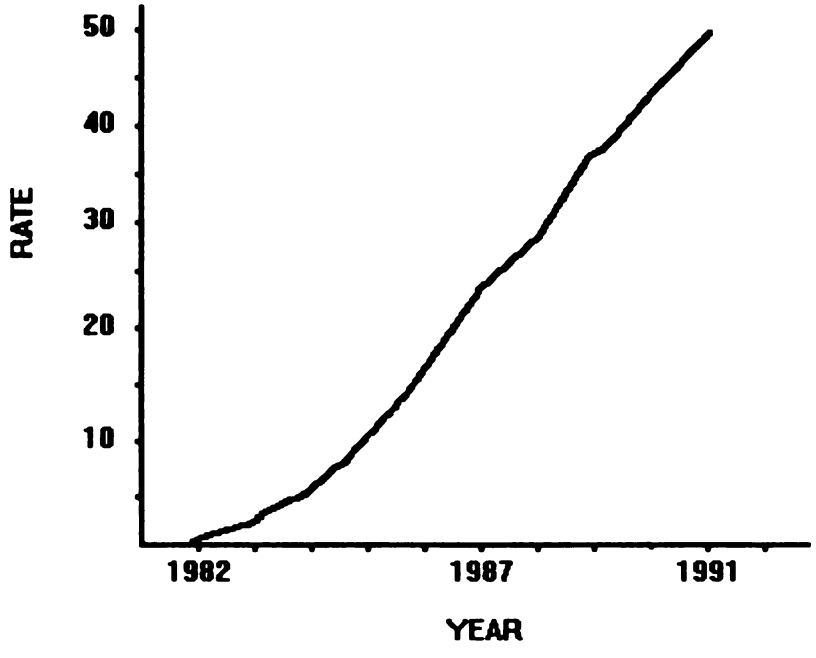
INTRODUCTION

The many advantages of connective tissue allografts have prompted an increase in their clinical use for ligament and meniscal replacement.[3,31,53] However, as the incidence of the Human Immunodeficiency Virus (HIV) within the general population is also rising,[12,34] the threat of viral transmission through connective tissue transplants has become an important issue.[3,53] Despite the serious nature of this issue, current views on the risk of retroviral transmission through connective tissues are based primarily on inference from case reports. To better define the potential for transplant-related HIV transmission, the risk of obtaining a connective tissue graft from an infected individual must first be established. Then, the ability of the connective tissue graft to transmit virus must be investigated.

The World Health Organization estimated that in mid-1993 there were over 1.0 million HIV-infected individuals in North America.[34] In 1991 alone, there were more than 29,000 HIV-related deaths in the United States and approximately 75% of these deaths occurred in the age group 25-44 years.[12] This age group, in which HIV is so prevalent (Figure 1), also represents the optimum source for connective tissue allografts.[5,53] The presence of HIV in this population has prompted guidelines for donor screening which include evaluation for risk factors and HIV antibody testing.[10] Unfortunately, the screening procedure may fail for several reasons and cases of HIV transmission from screened donors have been reported.[9,32,36,46] While other screening methods are available, they are not used routinely in all tissue banks.[3,53] Therefore, there is a definite risk that connective tissue allografts may be harvested from HIV-infected donors.

DEATH RATES* FOR HIV-RELATED DISEASE
FOR MEN AGED 24-44 YEARS: UNITED STATES (1982-1991)**



*deaths per 100,000 population
**adapted from CDC: MMWR 42:483, 1993

Figure 1: Annual death rates for HIV-related disease in men aged 25-44 years.

Although the risk of harvest from an infected donor has been established, the ability of musculoskeletal connective tissues to transmit HIV is not well-defined.[3] Because connective tissues are relatively acellular and avascular, these tissues have been considered unlikely to harbor HIV.[3,46,53] Connective tissue grafts have been associated with HIV transmission,[10,46] but all of these grafts contained cancellous bone and bone marrow. It has been suggested that the highly cellular cancellous bone and bone marrow elements were responsible for viral transmission and not the involved connective tissues.[3,53] Despite this theory, HIV has been identified in bone[6,42] and tendon[6] harvested from infected individuals, suggesting that transplantation of these tissues could result in viral transmission. There have been no studies which document retroviral transmission by connective tissues other than bone containing bone marrow.

If HIV can exist in connective tissue allografts, the ability to routinely and reliably sterilize all grafts would eliminate the risk of viral transmission. Several sterilization procedures are available for connective tissue grafts. Unfortunately, sterilization of grafts by various methods (i.e. ethylene oxide, gamma irradiation) may also harm the graft and/or the graft recipient.[53] While complete sterilization may not be possible, studies have suggested that routine freezing could minimize the risk of HIV transmission by decreasing the load of infectious virus in connective tissues allografts.[6,42] However, there have been no studies that show transplant-related infection would be inhibited by this process.

The lack of conclusive information concerning retroviral transmission in connective tissue allografts prompted an experimental study. The hypotheses of the study were as follows: 1) a retrovirus can be transmitted through the transplantation of connective tissue allografts other than bone, and 2) freezing the tissues will not decrease their ability to transmit virus.

To test the hypotheses, the study had two specific aims. The first was to transplant fresh and frozen connective tissue allografts (i.e. tendon, tendon-bone, and meniscus) from FeLV positive donors into specific pathogen-free (SPF) recipients. The second was to document viral transfer through serological testing including an enzyme-linked immunosorbent assay (ELISA) for viral antigen and an immunofluorescence assay for antibody against feline oncornavirus-associated cell membrane antigen (FOCMA).

The experimental model chosen for this study was the feline leukemia virus (FeLV), a retrovirus which has biological similarities to HIV including its replication cycle and activity.

SURVEY OF LITERATURE

Musculoskeletal Connective Tissue in Allotransplantation:

Musculoskeletal connective tissue allografts such as bone, tendon, and meniscus are used frequently in the reconstruction and replacement of severely injured orthopedic structures. Bone allografts are used extensively for spinal fusions, packing of bone cysts, fracture repair, and limb-sparing procedures.[53] Patellar tendon and patellar tendon-bone unit grafts are gaining wide acceptance for the reconstruction of the ruptured anterior cruciate ligament.[45,53] Likewise, the use of meniscal allografts is increasing for cases in which extensive damage makes repair of the meniscus impossible.[2] It is estimated that well-over 250,000 connective tissue allografts are transplanted yearly (Table 1).[28]

The advantages of connective tissue allografts over connective tissue autografts have been well-outlined.[21,29,31,45,53] First, the use of allografts does not require graft harvest from the clinical patient and thus lessens patient morbidity. Since only one surgical approach is necessary, anesthesia time, surgical manipulation of tissues, and risk of infection are decreased, while the integrity of the patient's normal tissues is left intact.[31,45] Secondly, allograft tissue banks allow storage of an abundant source of readily available connective tissues.[53] This is important when multiple grafts are required in a severely injured patient and when a previously placed autograft fails.[31] The availability of allografts is also beneficial for procedures in children who often have insufficient stores of available tissues for autografting.[31] Third, allografts are advantageous when autologous tissues are not available for replacement such as the meniscus.[2,31,53] Finally, in some cases, the cost of obtaining an allograft from a tissue bank may actually be less than the cost of autograft harvest.[53]

Table 1: Estimated number of tissue allografts used annually in the USA*

Cadaver Donor

Tissue	Number of Transplants
Bone	250,000
Cornea	36,000
Skin	5,000
Heart valve	2,000
Cartilage	1,000
Tendon	500
Fascia	500
Dura	500
Vein	100
Pericardium	100

Living Donor

Tissue	Number of Transplants
Red blood cells	12,000,000
Semen	172,000
Bone	5,000
Marrow	2,000
Embryo	2,500

* Adapted from: Jackson DW, Simon TM: Southern California Center for Sports Medicine, Orthopaedic Research Institute, Long Beach, CA.[28]

The advantages coupled with the promising results achieved with connective tissue allografts[2,45,53] have greatly increased their clinical application. However, connective tissue allografts do have disadvantages. The transplantation of any tissue carries the risk of immune response leading to rejection of the graft. However, many connective tissues are considered "immunologically privileged" with little risk of rejection. This is well-illustrated by the meniscus in which cells containing the major histocompatibility antigens are separated from the host's immune system by the matrix surrounding them.[2] In addition, the majority of bone and tendon grafts are frozen prior to implantation, a process which destroys cells and limits immunogenicity.[53] The most serious disadvantage of connective tissue allografts is the potential for transmission of infectious agents. Allografts have been associated with the transmission of several bacterial, fungal, mycobacterial and viral agents.[31] The threat of viral transmission, particularly the Human Immunodeficiency Virus (HIV-1), has raised serious questions about the use of musculoskeletal connective tissue allografts.

HIV Screening of Tissue Donors:

Routine testing of blood, organ, and tissue donors for HIV antibody was recommended after FDA approval of the assay in 1985.[8] In 1988, the Center for Disease Control, American Association of Tissue Banks, American Academy of Orthopedic Surgery, and the Federal Drug Administration met to draft guidelines for the prevention of HIV transmission in bone. The Public Health Service issued the recommendations for screening donors of bone and other allografts which include complete review of donors' medical records, interviewing living donors, and HIV antibody testing.[10] It is estimated that review of the donor's history for HIV risk factors and a self-deferral questionnaire may detect 80-90% of HIV carriers.[53,56] Similar donor screening has significantly reduced the risk of viral transmission through blood transfusion.[56]

However, recent reports indicate that donor screening may fail for several reasons.[9,46,56] While medical record review and interviewing donors may identify HIV risk factors, some HIV carriers do not have identifiable risk factors. Simonds et al.[46] documented a case in which an HIV- infected organ and tissue donor had no identifiable risk factors. Also, many tissue donors are unconscious or dead upon arrival at medical care facilities and are unable to respond to questions concerning HIV risk factors.[46,53]

In addition, routine HIV-antibody testing may not identify all HIV carriers. Existing antibody titers may be diluted by massive volumes of fluid or blood and yield false- negative results. In 1987, the CDC[9] reported the case of a severely traumatized man who received several blood transfusions. The man had no known risk factors and had tested negative for HIV during treatment. Two days later, the man died and his vital organs were harvested. A medical facility retested a blood sample submitted with one of the donated organs and received positive results. Unfortunately, three transplants had already been performed with organs from the same donor. Of even greater concern, antibody titers may not exist in HIV carriers at the time of tissue donation. A "window of seronegativity" between infection and development of antibody titer has been identified in HIV carriers. This has been documented as a source of false-negative HIV screens in donors of blood,[56] organs,[36] and other tissues.[32,46] Horsbourgh et al.[27] studied serial blood samples collected from 39 men before and after HIV seroconversion. Results of testing for antibody, antigen, and the presence of proviral DNA showed that approximately 70% of infected individuals develop an antibody titer within 4-6 weeks of infection and 90% will have a titer six months after infection. An HIV carrier tested prior to seroconversion could be selected as a donor.

Additional measures may be taken to improve donor screening. The CDC recommends antibody testing be performed only on serum taken prior to blood

transfusion.[11] The American Association of Tissue Banks now recommends that allografts be stored until surgical donors can be retested, six months after donation.[53] While these recommendations will reduce risk when a living donor is involved,they are not advantageous when cadaver donors are used. In such cases, further testing is necessary.[53]

An enzyme-linked immunosorbent assay (ELISA) for HIV antigen is available. Antigen testing may detect HIV infection at an earlier stage than does antibody testing.[4,53] Daar et al.[14] identified high levels of virus by culture and polymerase chain reaction (PCR) in serial plasma samples from four HIV carriers prior to seroconversion. The extremely high levels of virus found prior to seroconversion suggest that HIV infection is highly infectious at this time.[14,49] Therefore, it would appear that identification of the antigenemia would improve donor screening. However, antigen testing does have disadvantages which have limited its use in blood and tissue banks. The antigen test has a high false-positive rate which can eliminate a number of suitable donors.[53] In addition, studies have questioned its ability to identify additional cases of HIV in blood donors when compared to routine antibody testing. Busch et al.[7] found no cases of antigenemia without concurrent antibody titers while screening over 8,000 blood samples from high risk individuals. Daar et al.[14] and Stramer et al.[49] found that antigenemia may occur as little as one week prior to seroconversion. The short time between onset of antigenemia and seroconversion may account for the lack of a satistically significant increase in HIV detection by the antigen test.[14] Still, the test might show some increase in the number of detections when evaluating larger numbers of donors and when screening for risk factors is inadequate (i.e. cadaver tissue donors).

The polymerase chain reaction (PCR) also identifies early HIV infection.[53] PCR amplifies proviral DNA and may help detect low levels of HIV nucleic acids within 1 week of infection. Like the antigen test, PCR may not provide a statistically significant

increase in HIV detection in blood donor populations but may prove useful in evaluating cadaver tissue donors. Cost and technical difficulty have limited the use of this test for routine screening procedures.[53]

Until optimum donor screening methods are devised, there is a risk that HIV carriers will be included in donor populations. Buck et. al.[5] attempted to estimate the risk of obtaining an HIV infected bone graft through evaluation of the risk of failure for each screening test and combinations of screening tests. In a worst case scenario, the risk was 1:161 if only antibody testing was used to screen donors. If all available testing methods (i.e. antigen test, antibody assay, special lymph node exams, full autopsy, etc.) were used, the risk fell to 1:1.6 million.

Cases of HIV Transmission Through Bone Allografts:

There have been cases of HIV transmission through connective tissue allotransplantation. In 1988, the CDC reported the first case,[10] which involved a donation which occurred in 1984, prior to routine HIV antibody testing. The donor was a 52 year-old man who had a femoral head excised during a hip arthroplasty. The harvested bone was immediately frozen at -80°C and underwent no additional processing. After 24 days, the bone was donated for use in a woman undergoing spinal fusion for scoliosis. In 1986, the donor developed *Pneumocystis carnii* pneumonia (PCP) and tested positive for HIV. At that time, an interview of the donor revealed a history of intravenous drug use and review of previous medical records indicated a lymph node biopsy had been performed prior to donation of the femoral head. The donor died due to HIV-related disease in 1987. In 1988, the recipient developed generalized lymphadenopathy and PCP. The recipient was positive for HIV antibody. A review of the patient's history revealed no risk factors for HIV transmission except the bone transplant. While transplant-related HIV infection

had been previously reported, this was the first documented case of AIDS associated with transplanted tissue.[10]

In 1992, additional cases of HIV transmission through bone allografts were reported by Simonds et al..[46] The donor was a 22 year-old man who died of a gunshot wound in 1985. The man had no risk factors for HIV infection and two serum samples were ELISA negative for HIV antibody. A total of 4 organs and 54 tissues were harvested from this individual. The four recipients of the heart, liver and two kidneys all developed HIV infections. All of the musculoskeletal tissue allografts were fresh-frozen immediately after harvest. Four fresh-frozen allografts were transplanted without further processing. Of these grafts, three (two femoral head grafts and one bone-patellar tendon-bone graft) resulted in the transmission of HIV. A fresh-frozen proximal femur graft was used in a total hip arthroplasty and was reamed to remove medullary contents during the procedure. This graft did not result in HIV transmission. The remaining tissues underwent additional processing and many were eventually transplanted including the following: 25 pieces of freeze-dried, ethanol-treated bone, 4 freeze-dried soft tissues (2 fascia lata, 1 tendon, 1 ligament), and 3 pieces of freeze-dried, irradiated dura. None of these transplants resulted in HIV infection. Likewise, the transplantation of two fresh corneas did not result in HIV transmission. A total of seven recipients developed HIV infection.[46]

HIV Transmission and Connective Tissues:

Although there are documented cases of HIV transmission through bone allografts,[10,46] the ability of musculoskeletal connective tissues to transmit the virus has been questioned. It has been suggested that connective tissues are unlikely to harbor and transmit viruses because of their minimal cellularity and vascularity.[3,46,53] Because the connective tissue grafts associated with transplant-related HIV (3 fresh-frozen femoral head grafts and one fresh-frozen bone-patellar tendon-bone graft) contained cancellous

bone, it is believed that the highly cellular bone marrow associated with each graft was responsible for transmission.[3,46,53] Further supporting this theory, a bone graft harvested from one of the same donors but cleansed of marrow prior to transplant did not transmit HIV.[42] Based on this information, Tomford[53] suggests that bone marrow evacuation is the most important factor in reducing HIV transmission through bone grafts and that connective tissue grafts that do not contain bone have little potential for HIV transmission.

The relationship of cellularity to HIV transmission has been addressed in other tissues. In twelve cases,[32,43,46] transplantation of cornea from HIV-infected individuals has not resulted in infection. The lack of transmission has been attributed to the relative acellularity of cornea.[32,43,46] In the case report by Simonds et al.,[46] the transplantation of tendon, fascia, and ligament without attached bone from an HIV carrier did not result in viral transmission to recipients. However, these minimally cellular connective tissues were processed and sterilized prior to transplantation.[46] It is unknown whether the inherent nature of the connective tissues or the processing inhibited HIV transmission.[3]

Two studies suggest that minimally cellular tissues can harbor HIV. Buck et al.[6] cultured HIV from 3 out of 5 proximal tibial samples harvested from individuals within 13 hours of AIDS-related deaths. HIV was also cultured from one bone sample after 3 consecutive washes to remove bone marrow.[6] In addition, HIV was recovered from two out of four patellar tendons harvested from the same individuals.[6] Salzman et al.[42] demonstrated proviral DNA in bone obtained from two HIV positive autopsy specimens.

Allograft Tissue Processing and HIV:

Tissue processing and sterilization techniques used will depend upon the type of graft harvested and will vary between tissue banks. The different methods have been

reviewed.[3,53] Tissues are harvested during sterile surgical procedures on living donors and under sterile or non-sterile conditions from cadaver donors within 24 hours of death.[3,53] Allografts may be processed immediately after harvest and then stored frozen. Alternatively, may be frozen first and processed at a later date. During processing, harvested tissues are washed with antibiotic solutions.[3,53] The washing process may be augmented by high pressure lavage or ultrasonic agitation. Tomford[53] has suggested that the removal of bone marrow and blood during this washing step is the best method to reduce HIV transmission through bone grafts.

Alcohol rinses may also be used during processing. Spire et al.[48] documented the viricidal effect of alcohol on HIV *in vitro*. Alcohol at a concentration less than 10% had little effect on HIV as determined by reverse transcriptase activity. With 19% alcohol, reverse transcriptase activity was greatly reduced. Ultimately, 25% alcohol was recommended for disinfection of medical instruments. While alcohol may kill surface organisms on small bone chips, the ability of alcohol rinses to penetrate long bones has been questioned.[53]

Freezing is considered a simple and effective method of allograft storage. The goal of freezing is to stop enzyme activity within the graft and is usually done at -80°C.[53] Frozen tissues may be stored for several years with little effect on the tissues.[3,53] The freezing process will kill cells and thus decreases the immunogenicity of allografts.[53] It has been suggested that freezing and the subsequent cell death could also affect the ability of a graft to transmit virus.[3,6,42]

Two studies have addressed the effects of freezing on HIV in connective tissue allografts. Buck et al.[6] demonstrated that HIV could be cultured from 3 samples of tibia harvested from HIV-infected individuals. After freezing, the virus was recovered from

only one of the three samples which originally was culture positive. In addition, HIV was cultured from two patellar tendons before but not after freezing.[6] Salzman et al.[42] examined fresh-frozen bone from two autopsy patients who died from AIDS. The bone specimens were frozen, processed, and refrozen. While HIV was demonstrated in the bone marrow of one patient, the virus could not be cultured from bone samples. However, proviral DNA was detected in the bones by PCR. Salzman et al.[42] concluded that virus was present within the samples but was not infectious. The conclusions drawn from both studies were that freezing could decrease viral numbers significantly and possibly reduce the ability of the graft to transmit virus. However, the actual infectivity of the grafts was not investigated.[6,42]

Freeze-drying (lyophilization) of tissues may be performed after the initial freezing process. This process lowers the water content of grafts to less than 5% and allows the advantage of storage at room temperature.[3,53] Freeze-drying has been successfully used on several connective tissues and is the method of choice for storage of processed bone chips, wedges and powders.[53] Like fresh-frozen tissues, freeze-dried grafts will not contain live cells and this may affect the ability of the grafts to harbor virus. In the case report by Simonds et al.,[46] several pieces of freeze-dried, ethanol-treated bone did not result in HIV transmission to recipients. However, Tomford[53] suggests that the lack of transmission in this case was due partly to a low viral burden in the donor who was recently infected. Buck et al.[6] demonstrated that HIV could be cultured from freeze-dried bone.

Allograft Tissue Sterilization and HIV:

Ethylene oxide is an effective sterilizing agent for medical instruments as well as tissues.[53] However, the use of ethylene oxide in tissue banks is declining for several reasons. Ethylene oxide has little ability to penetrate substances and cannot adequately

sterilize large pieces of bone.[53] In addition, ethylene oxide sterilization produces toxic by-products which have shown adverse effects in clinical trials. In a long term follow-up study of patients with freeze-dried, ethylene oxide-sterilized bone-patellar tendon-bone allografts, Jackson et al.[29] reported chronic intraarticular reactions characterized by joint effusion and synovial inflammation. Patients responded favorably to removal of the grafts and ethylene chlorhydrin, a toxic product of ethylene oxide, was identified in one of these grafts. In another study, Roberts et al.[38] documented reactions including graft dissolution and femoral cyst formation in 22% of patients receiving freeze-dried, ethylene oxide-sterilized bone-patellar tendon-bone allografts. The poor results coupled with the environmental hazards associated with ethylene oxide sterilization have curtailed its use in many hospitals and tissue banks.[29,53]

Irradiation is a frequently used method of sterilization and, according to one study, the method most preferred by surgeons using allografts.[53] The most common source of radiation for allograft sterilization is the gamma irradiation emitted from Cobalt 60. The effects of irradiation are achieved by direct ionization of molecules or by other molecular reactions which result in ionization. Because it is non-particulate, gamma irradiation can readily penetrate substances such as thick cortical bone. Thus, it provides an effective and inexpensive way to sterilize allografts. Gamma irradiation is also safe for transplant recipients as it does not produce toxic residual compounds which can leach out of grafts into surrounding tissues. However, gamma irradiation does result in the production of free oxygen radicals which can cause tissue damage and may alter the graft.[53]

When using gamma irradiation, the major challenge is to find a dose that will effectively kill microorganisms without causing excessive damage to the graft.[53] Unfortunately, viruses tend to be less radiosensitive than bacteria.[53] Fideler et al.[19] evaluated bone-patellar tendon-bone grafts from 8 HIV-infected cadavers. Grafts were

treated with either 2.0 Mrad, 2.5 Mrad, 3.0 Mrad, or 4.0 Mrad of gamma irradiation. PCR was used to detect HIV. All grafts which received 2.0 Mrads were positive for viral DNA while one-half of those treated with 2.5 Mrads were positive. All grafts treated with 3.0 Mrads and 4.0 Mrads were negative. It was suggested that at least 3.0 MRads are needed to sterilize frozen bone-patellar tendon-bone grafts. Based on previous studies, Tomford calculated that 4.5 MRads are necessary to sterilize bone allografts.[53] These doses are much higher than the 2.5 MRads currently accepted by the International Atomic Energy Agency for sterilization of medical products and may also have deleterious effects on musculoskeletal connective tissue grafts.[19] Gibbons et al.[21] demonstrated significant reductions in maximum stress and maximum strain in goat bone-patellar tendon-bone grafts following irradiation with 3.0 Mrad doses. Doses over 3.0 Mrad have also been associated with decreased breaking strength in bone allografts.[35] The actual healing of an irradiated bone allograft within a patient may be minimally affected by irradiation.[53]

Feline Leukemia Virus:

Feline leukemia virus (FeLV) was first identified by Jarrett et al.[30] in several cats with lymphosarcoma. Like HIV, FeLV is a retrovirus, an RNA virus containing reverse transcriptase. Retrovirus gains entry to lymphocytes by first attaching to cell receptors. After penetrating the cell membrane, the virus uncoats and reverse transcriptase facilitates the production of double-stranded DNA. This DNA may become integrated as a provirus into the host cell genome, allowing replication whenever the host cell divides. In addition, a productive infection may result as the proviral DNA directs the host cell to produce viral RNA and proteins. These units are assembled and bud from the cell membrane as new viral particles.[18,22]

The pathogenesis of FeLV has been closely investigated and has been reviewed by many.[4,13,22] Natural FeLV infection is transmitted primarily through saliva and requires

close contact between individuals such as grooming or shared food dishes. The virus gains entry through mucus membranes and infects the lymphocytes in local lymphoid tissue. After several days to weeks, the infection may be cleared or can progress to involve the bone marrow [40] and intestinal epithelium.[13] Several host and viral factors play a role in determining if infection will be persistent including: dose of virus, route of infection, genotype of virus, age of host and immune status of host.[40] It has been estimated that 60-70% of naturally occurring FeLV infections will self-limit.[22,24] A self-limiting infection is generally accompanied by a humoral immune response. In persistently infected individuals, FeLV proliferates quickly in the bone marrow and intestinal epithelium. Peripheral leukocytes are infected, viremia develops, and the virus is disseminated.[22,40]

Several pathologic syndromes may result in the persistently infected cat. Infection is usually accompanied by a variable degree of immunosuppression often characterized by abnormalities in T-lymphocyte numbers and function. FeLV can also produce malignant transformation of cells by acting as a promoter of host cell oncogenes and by other mechanisms that are not completely defined. In addition, FeLV infection is related to several blood dyscrasias, enteropathies, and neurologic syndromes.[4,24]

In some animals infected with FeLV, transformed cells will develop a cell membrane antigen known as feline oncornavirus-associated cell membrane antigen (FOCMA). The origin of FOCMA is unclear.[4] FOCMA was first described by Essex et al.[16] as an antigen whose antibody will react on the cell membrane of altered feline lymphoma cells. A variety of antigens on the cell membrane of transformed cells may contribute to FOCMA.[33] Early work suggested that FOCMA was a tumor specific antigen but not a component of the virus.[22,47] FOCMA has also been described as an antigen that may be an expression of an endogenous envelope gene.[44] Vedbrat et al.[55]

suggest that FOCMA is a viral encoded antigen based on the ability of anti-FOCMA antibodies to bind viral particles and immunoprecipitate viral gp70 antigen. While the origin of FOCMA has been disputed, all authors agree that FOCMA can be associated with FeLV infection.[16,22,44,55] Cats may develop antibody titers against FOCMA.[16,22] Essex et al.[16,17] demonstrated that FOCMA antibody could protect kittens from malignancy induced by feline sarcoma virus. However, this protection does not appear to extend to other FeLV-related diseases. Swenson et al.[50] showed that cats with FOCMA titers had a higher prevalence of disease than those without titers. FOCMA antibody titers may be observed in the absence of viremia. Swenson et al.[50] demonstrated that 64% of cats in 2 FeLV positive households were seronegative for FeLV but had positive FOCMA titers. This may be an indication of past or latent infection.

Experimental infection may be induced using the Rickard strain of FeLV(FeLV-R). Rickard et al.[37] provided a detailed report of the pathology and growth of an experimental strain of FeLV in 1969. Hoover et al.[25] studied experimental infection with FeLV-R in cats of various ages. They found that cats inoculated intraperitoneally showed an age-related response to infection. In cats aged 2 months or less, 85% of infected cats became persistently viremic while 15% of infected cats over 4 months of age remained viremic. Rojko et al.[41] found a similar relationship demonstrating that 80% of immature cats and 14% of adult cats infected with FeLV-R became persistently viremic. Rojko et al.[41] also demonstrated that cats with self-limiting infection developed a transient lymphopenia within 2 weeks of exposure and had positive FOCMA antibody titers within 14-42 days of exposure. Hoover et al. documented FOCMA antibody titers in cats 10-13 weeks after intraperitoneal infection with FeLV-R. The cats in both of these studies developed thymic lymphoma several months after infection.[25,41]

FeLV Serological Testing:

Several reliable tests are available for the detection of FeLV. Of these, the immunofluorescent antibody assay (IFA) for intracellular p27 antigen is considered the standard due to its high sensitivity and specificity.[22,26] Hardy et al.[22] demonstrated that virus could be isolated in 118 out of 121 cats (97.5%) that test IFA positive. Infection will persist in 97% of IFA positive cats. The reliable prediction of persistent infection demonstrated by the IFA is due to the fact that it detects viral p27 core antigen within infected white blood cells. Therefore, the test detects virus when there is little chance that the infection will self-limit.[22]

The ELISA detects soluble p27 antigen in serum, plasma or blood. Soluble antigen is produced when the infection is localized to lymph nodes and during later viremic stages. Clearing of the localized infection can result in transiently positive ELISA results.[24] Hawks et. al.[23] confirmed the nearly 100% sensitivity and 100% specificity obtained by a commercial ELISA. False positive results were obtained in only 5 out of 375 cats tested.

An immunofluorescence test for antibody against FOCMA has been described.[16] The test uses a living cat lymphoma cell line (FL74) as a source of FOCMA antigen. Detection of a positive antibody titer against FOCMA indicates exposure to FeLV. Titers may be present as a result of current, past, or latent infection.[22,33]

FeLV as a Model for HIV:

In September 1989, the Global Programme on AIDS of the World Health Organization convened to review the different animal models for AIDS. The group developed recommendations for the ideal HIV animal model for the study of drugs and vaccines. Based on these recommendations, the ideal model was described as one which would use HIV itself, use a small animal with well-known genetics, immunology and

metabolism, target CD4+ lymphocytes and macrophages, involve blood, lymphoid tissues and the brain, have a mode of transmission mimicking that of HIV, including prenatal transmission, and produce disease with a short incubation period and resemble human AIDS.[15] In a review of animal models for HIV studies, Gardner et al.[20] suggest that viral genetic structure and patterns of pathogenesis are important criteria for model selection.

Based on these criteria, there is no perfect model for HIV studies.[15,20] Study of HIV itself can only be performed in the chimpanzee, gibbon ape, and rabbit. The chimpanzee is considered the ultimate model but cost and the endangered status of this species prohibit its frequent use as a model.[15] None of the species which are susceptible to HIV develop AIDS similar to that seen with human infection.[20] Several animal retroviruses found in non-human primates and other mammals have been studied as HIV models. They offer a safer alternate to the use of HIV. The use of mammals other than primates offers the benefits of additional safety, practicality, and lower cost. In devising a scheme for the optimal use of animal models for HIV, the World Health Organization Working Group encouraged the use of small animal models prior to studies in non-human primates.[15]

Gardner et al.[20] suggest that kittens experimentally infected with FeLV-R can be used successfully as a model for HIV. Swenson et al.[51] have used cats intravenously inoculated with FeLV-R to study phosphonoformate as a treatment for HIV infection. Taveres et al.[52] also used a clone of FeLV-R for AZT studies due to its similarity to HIV and the accurate assay systems available to detect the virus.

MATERIALS AND METHODS

PART I

In Part I of the study, fresh connective tissue allografts (i.e. tendon, tendon-bone, and meniscus) were transplanted from FeLV positive donors into SPF recipients. Cancellous bone grafts from the infected donors were also transplanted into SPF recipients and served as positive controls. Viral transfer was evaluated through serologic testing with an ELISA for viral antigen and a FOCMA antibody assay.

Source of Fresh Tissue Transplants:

Six, 8 to 10-week-old, SPF cats were obtained from a licensed dealer. On Week 0, a 3cc blood sample was obtained from the jugular vein of each cat. The cats were then inoculated intravenously with 0.1 ml of plasma containing $6x10^3$ focus forming units (FFU) of the Rickard strain of FeLV (donated by Dr. Lawrence Mathes, Department of Pathobiology, Ohio State University, Columbus, Ohio). After inoculation, the cats were housed under SPF conditions at University Laboratory Animal Resources, Michigan State University, East Lansing, Michigan.

Beginning two weeks after inoculation (Week 2), 3cc of blood were obtained weekly from each cat through Week 7. Plasma samples were tested for FeLV p27 antigen using an enzyme-linked immunosorbent assay (ELISA). Whole blood smears from samples obtained Week 7 were also tested with an immunofluorescence assay(IFA) for p27 antigen

in peripheral blood leukocytes. Approximately, eight weeks post-inoculation, four animals were selected as tissue donors.

Harvest of Fresh Tissue Transplants:

Each animal was anesthetized using mask induction, then intubated and maintained on halothane in oxygen. The right stifle of each donor was prepared for aseptic surgery. Anesthesia, surgical prep, and tissue harvest were performed in a surgical suite isolated from the recipient cats.

For tissue harvest, the skin was incised to reveal the right stifle joint. The patellar tendon was bisected longitudinally. One-half of the tendon was freed from all bony attachments to yield the patellar tendon graft. The other half was freed from its patellar attachment but left attached to the tibial crest. A small piece of the tibial crest was osteotomized with an osteotome. This created the patellar tendon-bone unit graft. The medial meniscus was then removed and freed from excess connective tissue. A hole was drilled in the proximal tibia using a 9/64" drill bit and a curette was used to harvest approximately 1.5 cc of cancellous bone. The patellar tendon, patellar tendon-bone unit, and meniscal grafts were rinsed with sterile saline to remove peripheral blood. Tissue grafts were wrapped in sterile, saline-moistened gauze and transported to the recipients within 15 minutes of harvest. After all tissues were harvested, the donor animals were euthanized with an intravenous injection of sodium pentobarbital while still under general anesthesia.

Recipients of Fresh Transplants:

Sixteen, 8 to 10-week-old, SPF cats, obtained from a licensed dealer, received fresh transplants. 3cc of blood were obtained from the jugular vein of each animal prior to surgery. Immediately after harvest from the donor, one fresh graft of either tendon (n=4),

tendon-bone (n=4), or meniscus (n=4) was placed in the right stifle joint of a recipient. In addition, four cats received cancellous bone grafts in the right tibia and would act as positive controls. Beginning two weeks after surgery (Week 2), 3cc blood samples were collected weekly from the jugular vein of each transplant recipient. Plasma samples through Week 6 were tested for FeLV p27 antigen using an ELISA. Plasma samples through Week 8 were tested for antibody against FOCMA by an immunofluorescence test. At the conclusion of the experiment, each recipient animal was euthanized with an intravenous overdose of sodium pentobarbital. The positive control group served as tissue donors for Part II of this study. From the remaining transplant recipients, previously transplanted tissues were harvested and underwent histological examination.

Surgical Procedures:

All recipients were anesthetized by mask induction. They were intubated with sterile endotracheal tubes and anesthesia was maintained with isoflurane in oxygen. The right stifle was then prepared for aseptic surgery. The recipient cats occupied a surgical suite separated from that occupied by the donor cats. Surgical approaches and transplant procedures were performed with instruments which were not exposed to the donor animals.

Cancellous bone grafts were placed through a small skin incision made cranial to the tibial attachment of the medial collateral ligament. A 9/64" Steinmann pin was used to drill a hole through the medial tibial cortex. Approximately 1.5cc of cancellous bone were packed into the medullary canal of the tibia. This graft also contained peripheral blood and bone marrow elements.

Remaining grafts were placed in the stifle through a lateral parapatellar approach. Patellar tendon and patellar tendon-bone unit grafts were placed through a tunnel created

in the lateral femoral condyle with a 9/64" Steinmann pin. Patellar tendon grafts were secured on the lateral aspect of the condyle with a single 4-0 polyglyconate suture. Patellar tendon-bone unit grafts were secured laterally by wedging the attached bone plug into the bone tunnel. The medial end of each tendon graft was allowed to hang free within the intercondylar notch of the joint. Meniscal grafts were placed free within the suprapatellar pouch. All joint capsule closures were performed with 4-0 polyglyconate suture in a simple interrupted pattern. Skin incisions were closed with 4-0 nylon in a simple interrupted pattern.

Post-operative Care:

Each recipient received .01mg buprinorphine subcutaneously to provide analgesia. The recipients were placed in separate cages in SPF housing at University Laboratory Animal Resources, Michigan State University, East Lansing, Michigan. Each recipient was inspected daily for signs of illness or rejection reaction. Skin sutures were removed two weeks after surgery.

Housing Under SPF Conditions:

All of the cats in this study were maintained under SPF conditions. This would insure that the SPF cats could not be infected by organisms which could easily invade their naive immune systems. In addition, the recipient cats were housed in individual cages. This would insure that transmission of FeLV by natural routes of infection would not occur between the recipient cats.

Entrance to the SPF unit was gained through a "clean " hallway. Prior to entry, personnel donned sterile jumpsuits, surgical caps, masks, and gloves. All equipment entering the unit was autoclaved or scrubbed with alcohol. None of the equipment had prior contact with any cats. Cats were transported to and from the surgical facility in

filtered carriers. All personnel, animals, or equipment exited the unit through a "dirty" hallway.

Serological Testing:

All blood samples were placed in 3cc EDTA tubes immediately after collection. The whole blood was centrifuged at 1800 rpm for 20 minutes. The plasma was removed, placed in plastic vials, and stored at -80 °C. Plasma samples were shipped on dry ice for serologic testing. All serological testing was performed through Dr. Larry Mathes, Department of Pathobiology, Ohio State University, Columbus, Ohio. The following is a description of the testing procedures performed.

An immunofluorescent assay (IFA) for FeLV p27 antigen was performed on Week 7 samples taken from the donor animals to confirm persistent infection. The test is performed on air-dried whole blood smears on microscope slides. The blood smear is fixed with either alcohol or acetone for three minutes. The fixative causes disruption of the white cell membrane exposing intracellular FeLV antigens. Rabbit anti-FeLV serum is added to the slide which is then incubated for one hour at 37° C. The slide is then washed. Fluorescein-conjugated goat antiserum to rabbit gamma globulin is then added. Slides are counter-stained with Evan's blue and then examined under a fluorescent microscope. FeLV infected cells exhibit an applegreen fluorescence.[22] Results are recorded as either positive or negative.

An enzyme-linked immunosorbent assay (ELISA) for FeLV p27 antigen was performed on all samples from the donor animals and on samples through Week 6 from the recipients. The ELISA used in this study (Viracheck/FeLV, Synbiotics Corp, San Diego, CA) detects viral antigen in plasma, serum, or whole blood. In the direct ELISA,[54] specific antibody is coated to a solid such as the well in a microtiter plate. The test sample

is added to the well and antigen, if present, will bind to the antibody. An antibody-enzyme conjugate is added to the well and binds to antigen. Unbound reactants are removed by a wash step and a substrate/chromogen mixture is then added. A color change results, allowing measurement of the extent of reaction. The color change may be measured in two ways. Qualitative measurement may be performed by visual inspection for obvious color change. Quantitative analysis may be performed by obtaining an optical density reading. With each set of samples, a positive control (from an FeLV infected cat) and a negative control (from an SPF cat) were run simultaneously. In the majority of samples, visual inspection and optical density correlate well. These results are recorded as a definite positive (+) or negative (-). In some cases, a color change may be evident and the optical density may be positive (as compared to controls) but only slightly above the optical density of samples considered negative by visual inspection. These results are recorded as weak positive (w+).

The plasma samples obtained through Week 8 were analyzed for antibody against feline oncornavirus-associated cell membrane antigen (FOCMA). Antibody titers were determined by an indirect membrane immunofluorescence test which has been described.[16] A cultured line of FeLV-infected lymphoblastoid cells supplies the antigen for this test. Cells from 5 day old cultures at a given density are washed twice with phosphate-buffered saline. Cells are then exposed to serial dilutions of cat serum for 30 minutes at 37°C and are again washed with phosphate-buffered saline. They are then exposed to a 1:20 dilution of fluorescein-conjugated goat anti-serum to cat IgG for 20 minutes at 37°C. After a final wash, the samples are resuspended in 50% glycerol and examined by fluorescent microscopy. End-point titers are the reciprocal of the highest serial dilution of serum which produces fluorescence in at least 50% of the cells. An antibody titer $\geq$1:16 to FOCMA is considered positive for exposure to FeLV.

Necropsy:

After euthanasia, the right stifle of each recipient in the patellar tendon, patellar tendon-bone, and meniscal transplant groups was inspected. In the patellar tendon and patellar tendon-bone unit groups, the distal one-third of the right femur was removed. In the meniscal transplant group, the menisci were identified and removed from the joint.

Histopathological Specimens:

All bone specimens were fixed in 10% buffered formalin and decalcified in 5% nitric acid and embedded in paraffin. Five-micron thick, transverse were cut in the transverse plane through the bone tunnel. Menisci were fixed in 10% buffered formalin. The menisci were embedded in paraffin and five-micron thick sections were cut in the horizontal plane. All sections were mounted on glass slides, stained with hematoxylin and eosin, and examined by light microscopy.

Statistical Analysis:

The onset of antigenemia was documented as present or absent for each recipient in each transplant group. The three transplant groups and the positive controls were then evaluated for differences between the groups in the incidence of antigenemia using a minimum Chi-square.

PART II

In Part II of the study, frozen connective tissue allografts (i.e.tendon, tendon-bone, and meniscus) were transplanted from FeLV positive donors into SPF recipients. Viral transfer was documented through an ELISA for antigen and the FOCMA antibody assay.

Source of Infected Tissues:

At the conclusion of PART I of this study, the positive control group was euthanized. Immediately after euthanasia, the right stifle of each of the four animals was prepared for aseptic tissue harvest. Patellar tendon, patellar tendon-bone unit, and a meniscus were harvested as described in PART I.

Processing of Infected Tissues:

The harvested grafts were rinsed in sterile saline and placed in separate sterile vials. Each vial was sealed in another vial which was then placed in a -80°C freezer. All samples were kept frozen for 10 weeks at which time they were transplanted into recipients.

Recipients of Frozen Tissue Transplants:

Twelve, 8 to 10-week-old, SPF cats received frozen tissue transplants. 3cc of blood were obtained from the jugular vein of each animal prior to surgery. One frozen graft of either tendon (n=4), tendon-bone (n=4), or meniscus (n=4) was placed in the right stifle joint of a recipient. Beginning two weeks after surgery (Week 2), 3cc blood samples were collected weekly from the jugular vein of each transplant recipient. Plasma samples through Week 6 were tested for FeLV p27 antigen using an ELISA. Plasma samples through Week 8 were tested for antibody against FOCMA by an immunofluorescence test. At the conclusion of the experiment, each recipient animal was euthanized with an intravenous overdose of sodium pentobarbital.

Surgical Procedures:

All recipients were anesthetized by mask induction. They were intubated with sterile endotracheal tubes and anesthesia was maintained with isoflurane in oxygen. The right stifle of each recipient was prepared for aseptic surgery.

The surgical procedures were identical to those used in the fresh transplant recipients. During the surgical approach, a vial containing the frozen implant was opened and the sterile vial was poured into a bowl containing room temperature saline. The sterile vial was opened and the graft removed. The graft placement and remaining surgery was identical to that described in PART I.

Post-operative Care and Housing:

The post-operative management and housing of frozen transplant recipients were identical to that described in PART I.

Serological Testing:

The ELISA for FeLV p27 antigen and immunofluorescence test for antibody against FOCMA were identical to those used in PART I.

Necropsy and Histological Specimens:

The necropsy procedures, specimen handling, and histologic evaluation were identical to those used in PART I.

Statistical Analysis:

The onset of antigenemia was documented as present or absent for each recipient in each transplant group. The three transplant groups were then evaluated for differences between the groups in the incidence of antigenemia using a minimum Chi-square. Finally, the onset of antigenemia was evaluated for differences between all fresh and frozen transplant groups.

RESULTS

Throughout the study, the cats were maintained under strict SPF conditions. There was no contact between donor and recipient cats before, during, or after surgery. The recipients cats did not have significant or prolonged contact with one another. None of the cats showed overt signs of clinical disease. All of the transplant recipients recovered from surgery without complications and none showed signs of graft rejection.

Tissue Donors:

The results of ELISA and IFA for the six donor animals are shown in Table 2. Only one cat (Donor #90) was weakly positive on Week 2 but all donor cats had positive results by Week 3. The positive test results persisted in all cats except two (Donor #76 and Donor #93) which showed occasional negative results. The IFA, used to verify persistent viremia, was positive in all but one cat (Donor #76). Based on their consistently positive results, four cats were selected as tissue donors (Donors #73, #82, #88, and #90).

Positive Controls:

The results of ELISA testing for the recipients of cancellous bone grafts, also containing bone marrow and peripheral blood, are shown in Table 3. The four recipients became ELISA positive two weeks after transplant and the antigenemia persisted through Week 6 at which time the testing was discontinued.

Table 2: Results of Serological Testing of Transplant Donors

	Results of ELISA for FeLV p27 Antigen* Week Post-transplantation							**Results of IFA for FeLV p27 Antigen**
Donor #	0	2	3	4	5	6	7	Week 7
73	-	-	w+	+	+	+	+	+
76	-	-	w+	+	w+	-	+	-
82	-	-	w+	+	+	+	+	+
88	-	-	w+	+	+	+	+	+
90	-	w+	+	+	+	+	+	+
93	-	-	w+	w+	w+	-	-	+

* + = positive; w+ = weak positive; - = negative

Table 3: Results of ELISA for FeLV p27 Antigen in Positive Controls

		Week Post-transplantation					
Recipient #	Donor #	0	2	3	4	5	6
315	88	-	+	+	+	+	+
244	82	-	+	+	+	+	+
607	73	-	+	+	+	+	+
242	90	-	+	+	w+	+	w+

* + = positive; w+ = weak positive; - = negative

Fresh Transplant Recipients:

The results of ELISA for FeLV p27 antigen for the fresh transplant recipients are shown in Table 4. All pre-operative (Week 0) plasma samples were negative. Positive ELISA results were detected in each group of recipients (tendon 3/4, tendon-bone 2/4, and meniscus 2/4). In all cases, the positive results were transient and the recipients were ELISA negative on Weeks 5 and 6. Minimum Chi-square analysis revealed no significant difference in the incidence of antigenemia among the four transplant groups.

The results for detection of antibody against FOCMA for fresh transplant recipients are shown in Table 5. All pre-operative samples were negative (<1:16). All animals showed some increase in titer by Week 3. While titers showed minor fluctuations, the overall trend was for titers to increase markedly and all animals were consistently positive from Week 5 throughout the conclusion of the study.

All fresh transplants were examined microscopically and showed similar results. There was normal incorporation of the tendon and tendon-bone unit grafts into the bony tunnels.[1] Occasional aggregations of mature lymphoid cells were evident on the margins of these grafts. Viable cells and blood vessels were apparent within the grafts (Figure 2). The meniscal grafts showed cellular proliferation along the free margins (Figure 3). There was no evidence of an active inflammatory response in any tissues examined.

Frozen Transplant Recipients:

The results for ELISA testing of frozen transplant recipients are shown in Table 6. All pre-operative (Week 0) plasma samples were negative for viral antigen. Positive ELISA results were detected in each group of recipients (tendon 3/4, tendon-bone 3/4, and meniscus 2/4). Results were transient in all animals except one frozen meniscal

Table 4 : Results of ELISA for FeLV p27 Antigen in Fresh Transplant Recipients*

Recipient #	Tissue Graft	Donor #	Week Post-transplantation 0	2	3	4	5	6
611	PT	90	-	-	w+	-	-	-
609	PT	88	-	w+	+	w+	-	-
313	PT	73	-	+	+	-	-	-
445	PT	82	-	-	-	-	-	-
608	PTB	82	-	+	w+	w+	-	-
444	PTB	73	-	+	-	-	-	-
245	PTB	88	-	-	-	-	-	-
316	PTB	90	-	-	-	-	-	-
610	M	90	-	-	+	-	-	-
314	M	82	-	-	w+	-	-	-
446	M	88	-	-	-	-	-	-
243	M	73	-	-	-	-	-	-

* PT = patellar tendon; PTB = patellar tendon - bone; M = meniscus
+ = positive; w+ = weak positive; - = negative

Table 5: Antibody Titers to FOCMA for Fresh Transplant Recipients

			Week Post-transplantation							
Recipient #	Graft	Donor #	0	2	3	4	5	6	7	8
611	PT	90	<4	<4	8	16	64	2048	4096	1024
609	PT	88	<4	64	512	128	512	1024	NA	NA
313	PT	73	<4	<4	256	256	16	128	512	2048
445	PT	82	<4	<4	64	256	8192	2048	4096	2048
608	PTB	82	<4	32	32	64	512	128	NA	NA
444	PTB	73	<4	<4	256	8	512	64	2048	2048
245	PTB	88	<4	8	256	256	4096	1024	512	2048
316	PTB	90	<4	<4	16	256	1024	512	2048	1024
610	M	90	<4	<4	8	8	1024	512	1024	512
314	M	82	<4	<4	128	256	4096	1024	4096	2048
446	M	88	<4	<4	256	128	4096	256	4096	1024
243	M	73	<4	8	128	256	1024	256	512	1024

* PT = patellar tendon; PTB = patellar tendon-bone; M = meniscus
+ = positive; w+ = weak positive; - = negative
NA = plasma not available for testing

Data are expressed as reciprocal of actual value
Antibody titers $\geq$16 are considered positive

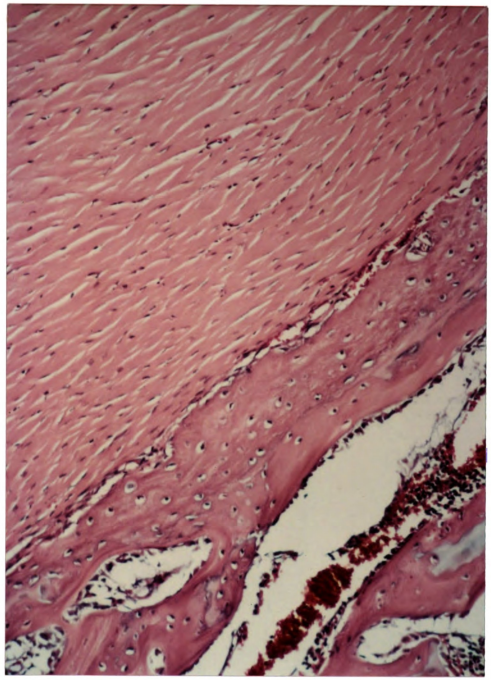


Figure 2: Photomicrograph of a longitudinal section of a fresh patellar tendon allograft within the femoral tunnel, 8 weeks following transplantation. (100x)

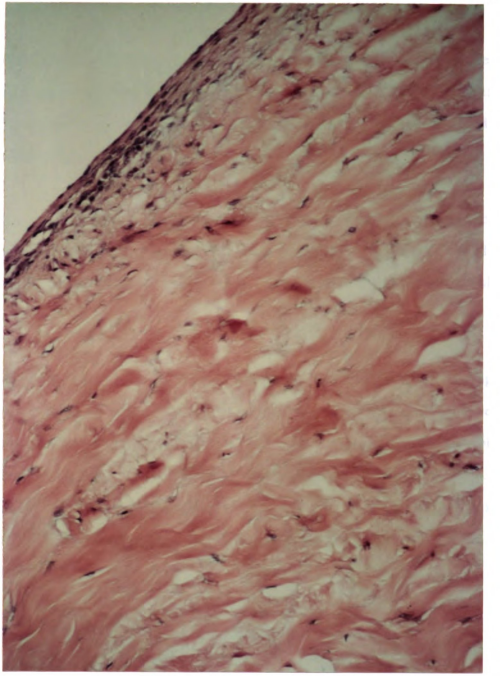


Figure 3: Photomicrograph of a transverse section of a fresh meniscal allograft, 8 weeks following transplantation. (100x)

transplant recipient (Recipient #724) which remained positive at the conclusion of the ELISA testing.

Minimum Chi-square analysis showed no significant difference in the incidence of antigenemia among the three transplant groups. In addition, minimum Chi-square analysis revealed no significant difference in the incidence of antigenemia between fresh and fresh-frozen transplant recipients.

The results of testing for antibody against FOCMA antigen in frozen transplant recipients are shown in Table 7. All animals except one (Recipient #724) demonstrated some increase in titer over the course of the study. Titers tended to rise slowly and showed fluctuations in several animals.

All transplants were examined microscopically and showed similar findings. All frozen tendon and tendon-bone unit grafts showed normal incorporation into the bone tunnel and evidence of cellular repopulation (Figure 4). All menisci contained viable cells within their substance and cellular proliferation was evident around the periphery of each graft (Figure 5). Similar repopulation of cells within fresh-frozen menisci has been described.[39] There was no evidence of active inflammatory response in any of the tissues examined.

Table 6: Results of ELISA for FeLV p27 Antigen in Frozen Transplant Recipients*

Recipient #	Tissue Graft	Donor #	Week Post-transplantation 0	2	3	4	5	6
708	PT	607	-	-	-	+	-	-
725	PT	315	-	-	w+	-	-	-
717	PT	242	-	-	w+	+	w+	-
720	PT	244	-	-	-	-	-	-
723	PTB	242	-	w+	+	-	-	-
UK1	PTB	244	-	w+	+	-	-	-
718	PTB	607	-	w+	-	-	w+	-
721	PTB	315	-	-	-	-	-	-
724	M	242	-	-	+	+	+	+
716	M	244	-	w+	w+	-	-	-
719	M	315	-	-	-	-	-	-
722	M	607	-	-	-	-	-	-

* PT = patellar tendon; PTB = patellar tendon - bone; M = meniscus
+ = positive; w+ = weak positive; - = negative

Table 7: Antibody Titers to FOCMA for Frozen Transplant Recipients

			Week Post-transplantation							
Recipient #	Graft	Donor #	0	2	3	4	5	6	7	8
708	PT	607	<4	<4	<4	<4	32	8	256	64
725	PT	315	<4	<4	<4	256	16	16	256	32
717	PT	242	<4	<4	<4	4	4	32	256	128
720	PT	244	<4	<4	256	32	1024	256	512	512
723	PTB	242	<4	<4	32	16	64	128	512	1024
UK1	PTB	244	<4	<4	32	32	<4	<4	8	<4
718	PTB	607	<4	<4	<4	16	64	<4	64	64
721	PTB	315	<4	8	16	<4	8	32	128	128
724	M	242	<4	<4	<4	<4	<4	<4	<4	<4
716	M	244	<4	<4	<4	16	256	16	256	128
719	M	315	<4	<4	<4	1024	512	128	1024	512
722	M	607	<4	<4	8	<4	256	8	256	256

* PT = patellar tendon; PTB = patellar tendon-bone; M = meniscus
+ = positive; w+ = weak positive; - = negative

Data are expressed as reciprocal of actual value
Antibody titers $\geq$16 are considered positive

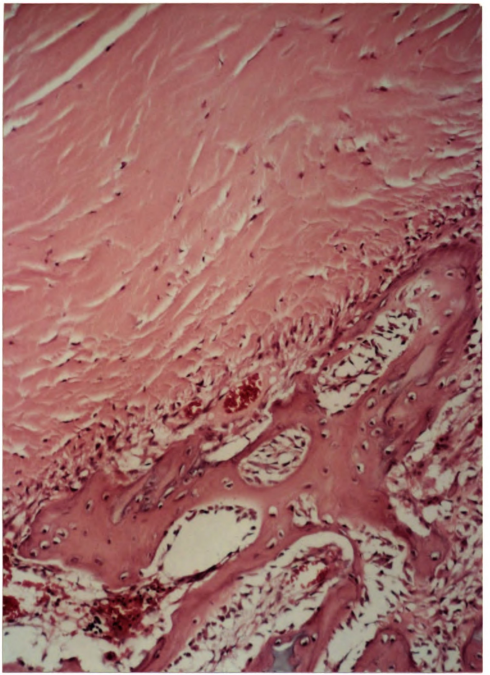


Figure 4: Photomicrograph of a longitudinal section of a frozen patellar tendon allograft within the femoral tunnel, 8 weeks following transplantation. (100x)

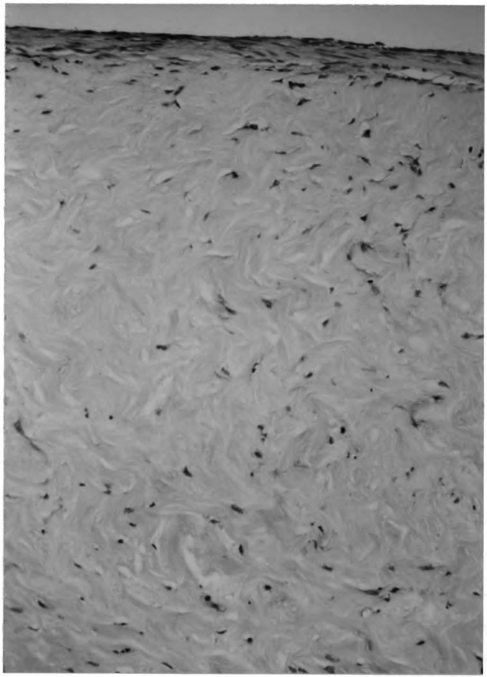


Figure 5: Photomicrograph of a transverse section of a frozen meniscal allograft, 8 weeks following transplantation. (100x)

DISCUSSION

Transplant-related HIV infection is a potentially fatal threat to recipients of connective tissue allografts. Yet, current beliefs about HIV transmission through connective tissue transplantation are based largely on speculation.[3,53] Previous studies have documented the presence of HIV in connective tissues[6,42] but there are no studies which address the infection of a recipient through these allografts. This study used an animal model to examine the potential for retroviral transmission in connective tissue allografts.

Using the criteria suggested by Gardner et al.,[20] the FeLV model was an appropriate representative of HIV for this study. FeLV and HIV are both retroviruses, sharing a similar structure and replication cycle.[18] FeLV also produces many of the same disease syndromes as HIV including blood dyscrasias, immunosuppression, and malignancies.[24]

The FeLV model provided several other advantages. As encouraged by the World Health Organization, the use of a small animal species preserved endangered primates from study.[15] The model also was less costly and safer than using non-human primates. As opposed to using a mouse model, the species selected was large enough to perform the surgical procedures and repeated blood collections necessary for the study. The use of the FeLV-R model in young, susceptible cats provided a reliable pattern of infection.[25,41] Therefore, it was necessary to inoculate only a small number of animals for use as tissue donors. Finally, there are several proven assays for detection of FeLV allowing documentation of viral transmission.[22]

Using the FeLV model, the study tested the hypotheses that a retrovirus similar to HIV would be transmitted through the transplantation of connective tissues other than bone and that freezing the tissue would not affect their ability to transmit virus. In this study, all fresh and frozen connective tissue grafts transmitted virus as evidenced by the results of antigen and/or antibody testing.

In the fresh transplant recipients, 7 out of 12 cats showed at least one positive ELISA result during the first four weeks of testing. However, all twelve cats were negative on Weeks 5 and 6. Although the positive results were transient, the high sensitivity and specificity of the ELISA[23] provide strong evidence that replicating virus was present in animals from each transplant recipient group.

The transient nature of the FeLV infection is not uncommon. After natural exposure, 60-70% of cats will have self-limiting infection.[24] These cats will show transient ELISA positive results. The duration of the FeLV infection is dependent on several factors such as immune status and size of inoculum.[40] In this study, the transient nature of the positive ELISA results may reflect the small inoculum provided by the transplants. In contrast, a persistent antigenemia was observed in the original tissue donors and the cancellous bone recipient groups which may have received a larger inoculum of virus.

The antibody titers to FOCMA in the fresh transplant group confirmed exposure to FeLV in all the recipients including those with negative ELISA results. Antibody titers against FOCMA observed in ELISA negative animals generally indicate either past or latent infection and have been reported after natural exposure.[50]

The ELISA results in the frozen transplant group varied only slightly from those seen in the fresh transplant group. Eight out of twelve cats had at least one ELISA positive result. One animal (Recipient #724) was persistently ELISA positive at the end of the study. As indicated above, the prolongation of infection in this cat could be due to several factors.[40] The response is most likely be due to an individual variation in immunocompetence, making this cat more susceptible to persistent infection.

The antibody titers against FOCMA in the frozen transplant recipients confirmed exposure to FeLV in most of the recipients including all those with negative ELISA results. However, the titers tended to develop more slowly and overall were lower than in the fresh transplant group. This could reflect the results of freezing and subsequent cell death. Without cells available, the virus would not replicate until local repopulation of the graft had taken place. In addition, some virus may have been destroyed when cells within the graft died. The persistently viremic recipient of a frozen meniscus (Recipient #724) did not develop an antibody titer to FOCMA, further supporting that this individual was immunocompromised.

Prior to this report, it was proposed that connective tissue allografts such as tendon and meniscus were unlikely to transmit retrovirus due to their minimal cellularity and vascularity.[3,53] In the reported cases of connective tissue transplant-related infections, it was postulated that cancellous bone and bone marrow elements were responsible for transmitting retrovirus.[3,46,53] However, the results of this study demonstrated no difference in the incidence of transmission between grafts that contained bone and those that did not contain bone (i.e. patellar tendon and meniscus). This suggests that retroviral transmission through connective tissues can be independent of tissue type and cellularity.

In addition, it would appear that the infectivity of the grafts was independent of graft location within the host. Patellar tendon and patellar tendon-bone allografts which showed normal incorporation into bony tunnels, as well as meniscal allografts which remained free in the joint, produced active infection in the recipients. This suggests that retroviral transmission is independent of vascular invasion and may be due to viral shedding by the tissues.

Routine freezing has been advocated as a process which may lower the chance of viral transmission in connective tissue allografts by decreasing viral load.[6,42] However, the results of this study indicate that retroviral transmission will occur readily through frozen connective tissues. While viral numbers may have decreased after freezing, enough retrovirus may survive the freezing process to allow infection to occur through the connective tissue allograft.

Although the risk of obtaining a connective tissue allograft from an HIV-infected donor is low,[5] the results of this study suggest that meniscal, patellar tendon, and patellar tendon-bone grafts from retrovirus-infected donors are capable of transmitting virus to a recipient. Freezing infected grafts did not appear to reduce viral transmission. Based on these findings, precautions to prevent transmission of HIV through connective tissue allotransplantation would appear prudent. Improvement of HIV screening methods for tissue and organ donors is an on-going battle which could further reduce but probably never eliminate the threat of transplant-related retroviral infection. In light of these facts, viricidal sterilization of all connective tissue allografts seems warranted.

CONCLUSIONS

1. In an animal model, a retrovirus similar to HIV was be transmitted from infected donors to specific pathogen-free recipients through fresh connective tissue allografts of patellar tendon, patellar tendon-bone, and meniscus.

2. Routine freezing of the connective tissues did not inhibit the transmission of the retrovirus.

3. Based on these results, viricidal sterilization of connective tissue allografts seems warranted.

FUTURE RESEARCH

The present study provides compelling evidence that retroviral transmission can occur through patellar tendon, patellar tendon-bone, and meniscus. However, several other connective tissues are routinely transplanted including heart valve, dura mater, costal cartilage, articular cartilage and many others.[28,31] Based on the information presented here, it is highly likely that some of these tissues could also transmit virus. Models similar to the one used in this study could supply information about the ability of these tissues to transmit retrovirus.

While routine freezing did not appear to inhibit viral transmission, other routine processing methods were not explored in this study. HIV has been cultured from freeze-dried tissues[6] but transmission through freeze-dried allografts has not occurred.[46] Freeze-dried tissues may be investigated using the present model. Likewise, the effect of various chemical agents such as alcohol rinses should be evaluated. In addition, further studies on freezing could document of the quantitative effect on viral load. The effect of a double freeze-thaw, commonly required during tissue processing, should be investigated. In vitro comparison of the animal retrovirus to HIV for susceptibility could add further validity to the animal models for viral transmission.

Based on the results of this study, viricidal sterilization of connective tissues appears warranted prior to transplantation. Gamma irradiation holds the most promise as an effective method of sterilization and further investigation of irradiation will eventually require an animal model. An *in vitro* comparison of the susceptibility of HIV versus an

animal retrovirus to irradiation should precede an *in vivo* study. When a range of effective doses of irradiation which minimally harm tissues has been established, the infectivity of irradiated grafts should be tested in an animal model.

FeLV provided a good model for retroviral transmission in this study. However, the Feline Immunodeficiency Virus may provide an even more appropriate model for some HIV comparisons. Like HIV, FIV belongs to the genus *Lentivirus* in the family *Retroviridae*.[18] This virus resembles HIV even more closely than FeLV and, along with the Simian Immunodeficiency Virus, is considered one of the best representatives of HIV.[20] This close relationship may be important when evaluating sterilization methods. FIV allows the continued use of cats, a readily available and safe species which is large enough for the surgical procedures required in transplant studies.

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