Introduction

The prevalence of allograft use in orthopaedics is on the rise. While the use of allograft bone and tendon is generally considered safe, the potential risk of disease transmission exists whenever tissue is transplanted from one individual to another (2,11). Rigorous donor screening, although not 100% effective, is currently thought to be the best available method to improve allograft safety (5,11). Despite these efforts, viral and bacterial transmission have been documented following transplantation of connective tissue allografts from infected donors (2,11). This has led to the development of a number of sterilization procedures designed to eliminate possible viral and/or bacterial transmission (5,11). While such procedures are effective in sterilizing connective tissue allografts in which cell viability is neither wanted nor needed for successful host incorporation and remodeling (i.e. bone, tendon), this is not the case for articular cartilage allografts. It has been shown that the functional outcome of articular cartilage transplants is directly related to the number of viable donors present at the time of transplantation (more live cells=better clinical outcome) (1). While the transmission of infectious retrovirus through the transplantation of bone and tendon allografts has been documented clinically and experimentally (2, 6), there is some debate in the literature regarding the ability of cartilage cells to harbor infectious retrovirus.

Our laboratory has developed in vivo and in vitro systems to test the infectivity of musculoskeletal allografts systemically infected with feline leukemia virus (FeLV) (10). Feline leukemia virus, a retrovirus with a structure and replication cycle similar to that of HIV, is widely used as an animal model for HIV. Previous studies have demonstrated that a variety of musculoskeletal tissues, including ligament and bone, transmit the FeLV retrovirus following allotransplantation (8). The purpose of this study was to determine the ability of intact cartilage fragments and isolated articular cartilage chondrocytes from FeLV systemically infected donors to transmit the retrovirus using our in vitro test system.

We hypothesize that articular cartilage fragments, but not isolated articular cartilage chondrocytes, from FeLV systemically infected cats are capable of transmitting the infectious retrovirus

Materials and Methods

Following institutional animal care and use approval, five, 10-week-old, specific pathogen free (SPF) cats were infected with the Rickard strain of the feline leukemia virus as previously described (8). The animals were monitored weekly over an 8 week period and all 5 animals tested positive for systemic FeLV infection by eight weeks. At that time, all 5 animals were humanely euthanized and fresh articular cartilage segments were harvested under sterile conditions using a ring curette and placed in culture media at 37°C. A portion of the cartilage fragments from each animal were kept intact while the remainder were used to isolate chondrocytes. To determine viral infectivity, intact cartilage fragments (≈1mm³) or isolated chondrocytes (expanded to passage 2) from each infected animal were co-cultured with feline embryonic fibroblast (FEA) cells. FEA cells are highly susceptible to FeLV infection and this in vitro co-culture system has been validated as a reliable assay for FeLV infectivity (10). At each passage, FeLV p27 antigen (an indicator of infectious virus) was measured in the co-culture media using an ELISA assay and FeLV (pro)viral nucleic acids were quantified by real time quantitative polymerase chain reaction (Q-PCR) using DNA extracted from the cells. In addition, fresh cartilage fragments, as well as isolated chondrocytes, were processed for immunohistochemical identification of the FeLV p27 antigen. Cartilage segments and isolated chondrocytes from SPF, non-FeLV infected cats were evaluated as negative controls.

Results

The FEA cells co-cultured with fresh cartilage fragments from each of the 5 FeLV-infected cats all demonstrated high levels of pro-viral, FeLV DNA indicating transmission of infective virus from articular cartilage allografts. In addition, media from the FEA–chondral fragment co-cultures from all 5 cats were positive for p27 antigen by the third passage indicating active viral replication. Co-cultures of FEA cells and isolated chondrocytes from the 5 FeLV-infected cats were negative for pro-viral FeLV DNA and p27 antigen.

Immunohistochemical staining of articular cartilage fragments from each of the 5 FeLV infected cats demonstrated the presence of p27 antigen throughout the extracellular matrix (Figure 1A). However, the p27 antigen did not appear to enter the chondrocytes. The lack of any positive p27 antigen staining of isolated chondrocytes provided additional evidence for absence of infectious virus in chondrocytes (Figure 1B).

Discussion/Conclusion

A previous study suggested that because cartilage from HIV positive donors was negative for pro-viral HIV-1 DNA, transmission of the HIV retrovirus through transplantation of chondral allografts was improbable (4). Another study that documented the inability of chondrocytes to be infected by HIV in vitro provided further support for this conclusion (3). However, viral transmission (infection) could result from infectious virus particles that made their way into the extracellular matrix of the articular cartilage from external sources (such as the synovium) rather than from the articular cartilage chondrocytes themselves. The results of the current study demonstrate that while pro-viral FeLV DNA could not be identified in isolated chondrocytes from FeLV infected animals, intact cartilage fragments from these same animals contained large amounts of p27 antigen in their extracellular matrix and were capable of transmitting infectious virus in our in vitro test system. Retroviral transmission via acellular mechanisms is well known. Prior to institution of viral inactivation procedures, hemophiliac patients were at extremely high risk of acquiring HIV infection through the transfusion of clotting factor proteins (6,7). Likewise, only 0.1ml of plasma from an infected animal can readily transmit FeLV to cats (9).

The results of the current study support the conclusion that articular cartilage fragments can readily transmit infectious retrovirus even in the absence of pro-viral DNA within articular cartilage chondrocytes. In addition, because donor cell viability markedly improves the long-term function of chondral allografts (thus, precluding the use of sterilization procedures currently employed for some tendon and bone allografts), these results also underscore the importance of rigorous donor screening when considering the use of articular cartilage allografts.

References: