Introduction  In recent years several approaches in gene therapy to the joint have been investigated. Although direct, in vivo transduction with some viral vectors is reported to reach high transduction efficiency in replicating and non-replicating cells, safety considerations suggest cell mediated approaches may be preferable.

Indeed, there is high evidence in a present human clinical trial for the treatment of rheumatoid arthritis that the use of retroviral transduced synovial fibroblasts is feasible for delivering interleukin-1 receptor antagonist protein (IRAP) to the arthritic joint. A transgene expression can be detected for up to six weeks after transplantation of synovial cells\(^1\). Cell transplantation in general has been limited by the survival of the injected cells in the tissue. In fact, it has been reported that major cell death occurs quickly following transplantation\(^2\). The aim of our study is to compare the initial decline of in vivo transgene expression of different cell types, i.e. muscle derived cells, skin fibroblasts and synovial cells, in a rabbit animal model. A high cell survival rate following cell mediated gene transfer is a prerequisite of persistent transgene expression in chronic disabling diseases such as rheumatoid arthritis.

Methods  In vitro IRAP Expression We seeded \(10^6\) cells of each culture in the respective well of a six well dish. Twenty-four hours after seeding, we performed a transduction with an adenovirus carrying the gene encoding interleukin 1 receptor antagonist protein at an MOI of 50. The supernatant was collected and replaced by fresh medium every 24 hours for 3 days. IRAP levels in the supernatant were quantified with a commercially available ELISA (Quantikine, R&D Systems). Harvesting of the Cells For the in vivo experiments, we harvested and isolated muscle derived cells and skin fibroblasts from the same rabbit. Additionally, we used an immortalized cell line of synovial cells (HIG). Cell transplantation Twelve hours after transduction the respective cells were harvested by trypsinization, counted, spun down (3500 rpm for 5 min), homogenized in 0.5 ml Hank’s balanced salt solution (HBSS), and immediately cooled on wet ice. Cells were injected into the knee joints of 3 month old New Zealand White rabbits (2.5 lbs.) within 10 minutes. In vivo Luciferase (Marker-Gene) Expression In a following set of experiments, we followed the transgene marker (luciferase) expression of synovial and muscle derived cells in the rabbit knee joint. The cells were transduced with an adenovirus carrying the luciferase gene and \(3x10^6\) cells were injected into the respective joint. The joint fluid was harvested 0, 6, 12, 24, 48 hours after injection by a joint lavage with 0.9% NaCl solution. We quantified the transgene expression with an assay for luciferase chemoluminescence. In vivo IRAP Expression For the in vivo expression of the IRAP, we transduced skin fibroblasts, muscle derived cells, and HIG synovial fibroblasts with an adenovirus carrying the gene encoding IRAP at an MOI of 50. \(3x10^6\) cells were injected into the respective joint. A sham control group received an injection of HBSS. The transgene expression was quantified at 0, 24, 48, 72 and 120 hours after intraarticular injection into rabbit knee joints. We quantified IRAP expression in the joint lavage fluid, performed a cell count, and evaluated smears from the lavage fluid microscopically. All experiments were approved by the authors’ institution, following the guidelines laid out by the USA Department of Health.

Results  In vitro IRAP Expression Among the investigated cell types, the highest IRAP concentrations were detected with skin fibroblasts at 24, 48 and 72 hours post transduction (Fig. 1). All investigated cell types maintained or increased their transgene expression over 72 hours. In vivo Luciferase Expression Concerning the luciferase assay, we observed a major decline of transgene expression for muscle derived cells and synovial cells within the first 12-24 hours (Fig. 2). In vivo IRAP Expression In vivo IRAP expression confirmed the results of the marker-gene expression. A rapid decline in transgene protein expression was observed within the first 24 hours following implantation in vivo. Five days after injection of the allogenic cells, there was hardly any persistence of IRAP expression (Fig. 3). For the first 5 days, muscle derived cells showed a less rapid decline in transgene expression than skin fibroblasts and HIG cells. Synovial Fluid Smears We detected in the skin cell, muscle derived cell, and HIG cell injected knees a high amount of neutrophils in the synovial fluid smears.

In groups treated with a cell injection, we observed clusters of injected cells with granulocytes (Figure 4). Cell mediated gene therapy has already proven to reach sustained intraarticular levels of therapeutic proteins such as IRAP\(^3\). Similar to results in cell mediated gene therapy to muscle tissue, a major decline in transgene expression occurs within the first 24 hours.\(^2\) In contrast to the in vitro results, the in vivo transgene expression decreased significantly within the first 3 days in all investigated cell types. Microscopic analysis of the synovial fluid confirmed a granulocytic inflammatory response of the host with eosinophilic granulocytes and clusters of neutrophils around cell debris. The anti-inflammatory effect of IRAP in the respective joint may lead to a slightly longer transgene expression than the luciferase marker-gene.

The early decline in transgene expression and the granulocytic infiltration hint at an early unspecific inflammatory reaction. Further experiments in non-immunocompetent animals and with autologous cells will show whether the inflammation induces the cell death or the inflammation is a response to the cell death. Although crucial for long-term transgene expression, cell selection does not play a key role in the early cell survival after intraarticular transplantation. However, the use of cells from peripheal tissues, including dermal fibroblasts and myoblasts, may decrease the invasiveness of cell isolation.

Reference

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