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Commentary

**Ex vivo gene transfer in the years to come**

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**Abstract**

Synovial fibroblasts (SFs) have become a major target for ex vivo gene transfer in rheumatoid arthritis (RA), but efficient transduction of RA-SFs still is a major problem. The low proliferation rate and heterogeneity of RA-SFs, together with their lack of highly specific surface receptors, have hampered a more extensive application of this technique. Improving transduction protocols with conventional viral vectors, therefore, as well as developing novel strategies, such as alternative target cells, and novel delivery systems constitute a major challenge. Recent progress in this field will lead to the achievement of high transgene expression, and will facilitate the use of gene transfer in human trials.

**Keywords:** ex vivo approach, gene therapy, rheumatoid arthritis, viral vector

**Introduction**

It is now well established that successful approaches to gene transfer in rheumatoid arthritis (RA) require three main questions to be answered. What genes or gene constructs should be used? Which cells should be targeted? How should the transgenes be delivered to the respective target cells [1]? These questions appear to be distinct, but in fact are linked closely to one another. Gene transfer designed to alter signalling pathways or the expression of matrix degrading enzymes will thus require in vivo approaches that ensure transduction of the majority of the synovial (lining) cells [2]. In contrast, gene transfer of secreted proteins can be done successfully, also ex vivo, where only a limited number of synovial cells is transduced [3]. Provided the expression levels of the soluble factors are high enough, re-injection of the transduced cells into the joint will give significant effects. In theory, these effects are limited only by the life span of the cell or may even exceed it when constructs are used that result in integration into the genome.

Ex vivo gene transfer of cytokines or (soluble) cytokine receptors/antagonists to synovial fibroblasts (SFs) has been suggested as a promising approach to interfere with synovial inflammation [4]. This is based both on the understanding that inflammatory cytokines contribute significantly to the pathogenesis of disease [5–8] and on our growing awareness of SFs as one major cell type involved in the destructive process of RA [9,10]. Fibroblast-like cells make up the largest population of resident cells in the synovium and can be grown easily in vitro. When their cellular properties are considered, however, RA-SFs are far from being ideal for gene transfer. They lack the expression of highly specific surface markers and their proliferation rate is relatively low. Also, RA-SFs are not a homogeneous population of cells, but several lines of

CIA = collagen-induced arthritis; Fv = variable fragment (of an antibody); IL = interleukin; IL-1Ra = interleukin-1 receptor antagonist; RA = rheumatoid arthritis; SF = synovial fibroblast; TCR = T-cell receptor.
use of retroviral delivery systems with RA-SFs
For *ex vivo* gene transfer of RA-SFs, retroviral vectors have been used frequently. This is because retroviruses are easy to produce and, through integration of their genetic information into the host genome, they can achieve long-lasting expression of the transgene. Apart from the duration of expression, however, actual levels of transgene expression are critical for successful gene therapy approaches. These are determined by the promoter that is used for transgene expression and the actual transduction efficacy (i.e., the percentage of cells that can be transduced from a certain population). Retroviruses can transduce only dividing cells, which limits their use with RA-SFs. For *in vitro* experiments or animal studies, several retroviral vectors have been developed that contain antibiotic resistance genes and allow for the selection of successfully transduced cells. Such vectors, however, cannot be used for human trials.

Several techniques [11] have been developed that allow the enhancement of the efficacy of retroviral transduction: the extension of the time of transduction and repeated transductions [12]; the use of different cationic agents [13]; centrifugation [14]; and flow-through techniques [15]. While these techniques are not new per se, little is known about their application in the retroviral transduction of human RA-SFs. In this context, del Vecchio and colleagues recently reported their experience in using different strategies to optimise the transduction efficacy of RA-SFs [16]. They demonstrated that the application of a combination of methods may enhance significantly the production of the interleukin-1 receptor antagonist (IL-1Ra) in RA-SFs that were infected with an MFG vector-based retroviral construct carrying the IL-1Ra gene. This is of interest, because their experience is taken directly from the first human clinical trial of arthritis gene therapy approved by the Recombinant DNA Advisory Committee of the National Institutes of Health in the United States and the US Food and Drug Administration [17]. Consequently, the paper raises a number of issues that are of importance, not only for the reported human trial but also for future clinical studies using gene transfer in RA. They demonstrated that virus titers of about \(10^8\) infectious particles per millilitre, together with the use of the lipopolyamine, dioctadecylamidoglycylspermine, instead of the conventional Polybrene, significantly enhance the levels of IL-1Ra produced by transduced RA-SFs. Centrifugation of the cells during transduction or flow-through resulted in a further dramatic effect; the highest levels were nearly \(1\ \mu\text{g}\) of IL-1Ra per \(10^6\) cells. This is of importance because inhibition of IL-1 by IL-1Ra requires a large molar excess that is difficult to achieve by pharmaceutical approaches. In the report, the exact number of successfully transduced cells was not investigated, but the increase in the production of IL-1Ra is probably due to an increase in transduction efficacy. Although the authors do not show the data of all combinations and give no specific recommendation as to which combination of techniques should be used, it may be speculated that the use of high viral titers, in combination with dioctadecylamidoglycylspermine and centrifugation/flow-through techniques, may result in the best transduction efficacy and, thus, in highest levels of proteins.

Further studies will be needed to see whether the described levels of IL-1Ra production can be maintained over time. It has been reported [8,18,19], that the severe combined immunodeficient mouse co-implantation model of RA may serve as a useful tool to investigate the long-term effects of modified transduction protocols on the expression and biological consequences of gene transfer. In this model, the question of whether cryoconservation affects the transduction of RA-SFs could also be answered.

Alternative *ex vivo* approaches for RA
Despite the advances in improving retroviral transduction of RA-SFs, several alternative strategies are being developed that permit efficient *ex vivo* gene transfer of secreted, anti-inflammatory molecules. As discussed at the Second International Meeting on Gene and Cell Therapies of Arthritis and Related Disorders in Montpellier [20], the efficacy of *ex vivo* approaches may be increased when cell types other than RA-SFs are used. Such cells may not be specific for the rheumatoid synovium but, following genetic modification, accumulate in the RA joint and secrete anti-inflammatory molecules.

Several groups have proposed to use genetically engineered T cells that would home specifically into arthritic joints and deliver anti-inflammatory molecules to the site of inflammation. Nakajima and colleagues thus used type II-collagen-specific CD4+ T cells that were transduced with retroviruses to express green fluorescent protein and they demonstrated that these cells accumulated and remained in the inflamed joints of collagen-induced arthritis (CIA) [21]. Using this method to deliver IL-12 after primary immunization, the authors found significant inhibition in the development of CIA. Chernajovsky and co-workers used retroviral delivery to engineer mouse T cells expressing a chimeric receptor that included the single chain Fv domain of the anti-type-II-collagen monoclonal antibody, C2, the...
hinge region of CD8-α and the transmembrane and cytoplasmic domains of TCR-ζ [22]. As suggested, such T cells can be used as gene carriers in arthritis.

Other approaches include the use of mesenchymal stem cells, dendritic cells or muscle cells rather than RA-SFs. It has been demonstrated that myoblasts can be transduced more efficiently than synovial cells, and that after intra-articular injection they adhere to different structures in the joint [23]. Recently, dendritic cells (derived from bone marrow), transduced with retroviral vectors carrying the IL-4 gene, were applied successfully to reduce the incidence of CIA in mice, as well as to suppress established disease in these animals [24]. In this approach, however, the effects were attributed to the migration of the genetically engineered dendritic cells to lymphoid organs and subsequent modulation of the T cell repertoire, rather than to an intra-articular expression of the transgene.

Apart from the use of alternative target cells, novel viral vectors are under development that may also be used for efficient ex vivo gene transfer. These include vectors derived from adeno-associated viruses as well as lentiviral vectors.

**Conclusions**

Despite a continuous increase of efforts to establish in vivo protocols for gene transfer of arthritis, ex vivo gene transfer of secreted factors such as cytokines or cytokine receptors/antagonists bear a great potential, and are the most advanced when it comes to possible clinical applications. Further modifications of protocols will help to increase the transduction efficacy and facilitate the establishment of clinical trials that are based on known vector systems. In addition, the use of alternative target cells will take advantage of the complexity of disease and may result in a site-specific delivery of ex vivo transduced but systemically applied cells.

**References**