Gene transfer in rheumatoid arthritis: A novel therapeutic approach


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Rheumatoid arthritis (RA) is a chronic systemic disorder of unknown etiology that is characterized by progressive joint destruction. Several novel strategies in the treatment of RA have evolved during the last few years. New insights into the molecular mechanisms involved in the pathophysiology of RA as well as advances in biotechnology not only have led to the development of highly specific biological agents for the treatment of RA (1), but also have given rise to the concept of gene transfer as a potential therapeutic approach to RA (2). Gene transfer, defined as the insertion of genetic information into living cells, can be applied with experimental or therapeutic aims. While in the past gene transfer was predominantly used to investigate cellular mechanisms involved in the pathogenesis of RA (3), the focus is shifting continuously to therapeutic interventions (2).

The feasibility of the strategy to selectively inhibit pathways involved in the pathophysiology of RA has been proven by the successful clinical application of biological agents such as inhibitors of tumor necrosis factor alpha (TNF-α) (4). However, the clinical effectiveness of such biologics is limited by their protein properties. They are not orally active and tend to be cleared rapidly after injection. The concept of gene transfer offers the opportunity to overcome this disadvantage by the specific delivery and long-term expression of therapeutic molecules in the target tissues. Nevertheless, several questions about the practical use of gene transfer in RA have not been answered sufficiently so far (5).

The efficacy of gene transfer is determined predominantly by the delivery strategy used. Therefore, the development of appropriate delivery systems is a critical step toward successful gene transfer. In this context, an interesting article by P.H. Goossens et al. addressing the efficacy of different promotors in the synovium of rheus monkeys with collagen induced arthritis is published in this issue (6).

In general, cells can be transfected either ex vivo or in vitro. For ex vivo transfer, the cells are removed prior to transfection and re-implanted afterwards. For this method, retroviral vectors still constitute the method of choice (Table I). Although transfection with retroviral-based vectors is restricted to dividing cells, the ex vivo procedure permits the selection of transfected cells and the growing of them under selective conditions before re-implantation into the joints. Therefore, the number and properties of the transfected cells can be determined before re-implantation.

Another characteristic feature of retroviral vectors is the genomic integration of the transferred gene (Table I). Since the integration is not site-specific, it bears the potential risk of insertional mutagenesis. However, due to the high transfection efficacy on isolated synovial cells and the possibility of analysing the cells in culture before re-implantation, ex vivo retroviral mediated gene transfer has been used for several experimental gene transfer studies in animals (7, 8) as well as for the first clinical trial of gene transfer in RA (9).

In contrast to retroviruses, adenoviruses are able to transfect non-dividing cells and show a high transfection efficacy (Table I). Therefore, adenovirus-based vectors have been used frequently for in

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<td>Retrovirus</td>
<td>Large packaging capacity (7 - 10 kb)</td>
<td>Require target cells in division</td>
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<td>No viral proteins produced</td>
<td>Risk of insertional mutagenesis</td>
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vivo gene transfer. Since adenovirus-based vectors are applied to the joints directly, the number and type of transfected cells can only be estimated. To determine the cells transfected with an adenoviral vector, Goossens et al. analyzed the cells of adenoviral-treated joints by immunohistochemistry. In accordance with previous reports, they found CD68-positive as well as CD68-negative cells transfected, indicating that adenoviral transfection is not restricted to one specific cell type. However, transfected cells were restricted to the synovium. No cells of the adjacent bone, cartilage, muscle or adipose tissues were found to express the transgene. The detected marker gene expression in peri-vascular regions, together with data from other studies (10), indicates that inflammatory cells could be efficiently transfected by adenoviral vectors in RA synovium.

Apart from the delivery system, the efficacy of transgene expression depends on the promoter used. In analogy to the delivery systems, different promotors have been utilized for gene transfer. In general, different types of promotors can be distinguished. On the one hand, there are promotors which mediate a constitutive and strong expression of the transgene in all transfected cells, while on the other hand tissue-specific and inducible promotors result in an expression of the transgene only in specific cells. For a constitutive expression either promotors of ubiquitous expressed genes like beta-actin or GAPDH have been used (promotors of housekeeping genes) or strong active viral promotors such as cytomegalovirus (CMV) or Simian virus 40 (SV40).

In their study, Goossens et al. compared the efficacy of the CMV and MLP promotors for transgene expression in cultured RA synovial fibroblasts and RA synovium. The CMV promotor was derived from the cytomegalovirus, whereas the MLP promotor was an adenoviral encoded promotor. Although both of them are of viral origin and belong to the constitutive active promotors, there were remarkable differences in transgene expression by these vectors. In comparison to the MLP promotor, the CMV driven transgene expression was significantly higher in both, in vitro and in vivo. Therefore, using a CMV promotor, the same expression level of transgene protein can be achieved using less of the vector. This is of special importance for adenoviral gene transfer, as the adenoviral-induced immunoreaction is dose-dependent. The high immunogenicity of adenoviral promotors is one of the limiting factors for adenoviral gene transfer. In addition, the direct, viral gene expression independent induction of signaling cascades by adenoviruses in synoviocytes affect the utility of adenoviral vectors for the treatment of arthritis (11). To improve adenoviral vector systems, the function of different adenoviral promotors as well as their interactions with the immune system were investigated. It has been shown that some adenoviral promotors have an immunomodulatory rather than an immunogenic effect (12). Adenoviral promotors, in which immunomodulatory genes were removed to increase the space for the inserted transgene, induced an increased immunoreaction. To investigate promotor and adenoviral vector-dependent inflammation, Goossens et al. scored the inflammation of different treated and untreated joints. Interestingly, they did not find any differences in the score of inflammation. The latter fact indicates that neither the adenoviral promotor, which contained the immunomodulatory E3 sequence, nor the CMV and MLP promotor induced inflammation. However, the authors did not investigate the induction of immunoreactions by repetitive administration of the vector. Due to their transient expression, repetitive administration is needed to achieve a sustained expression of the transgene in adenoviral transfected cells. Therefore, the induction of an immune reaction still constitutes a major problem for long-term treatment. The presence of neutralizing antibodies against adenovirus type 5, caused by naturally aquired infections, is another problem associated with the use of adenovirus-based promotors (13).

The construction of chimeric adenoviral promotors which lack surface proteins of adenovirus type 5 represents an efficient approach to solve this problem. As this problem is difficult to study in animal models, human trials are needed to evaluate the beneficial effects of new generation vectors.

Although optimal delivery systems for gene therapy of RA are not yet available, gene transfer has been shown to be a powerful and feasible therapeutic approach (14). Since gene transfer is one of the most rapidly developing and promising fields in biomedical research, further advances can be expected in the coming years. To develop novel strategies for the treatment of RA, gene transfer studies utilizing the humanized SCID mouse model are aimed at the following goals (5, 14, 15):
- To target the signalling pathways in the activation of synovial cells;
- To identify the pivotal matrix-degrading enzymes, such as matrix-metalloproteinases (collagenases) and/or cysteinproteinases (cathepsins)
- To stimulate apoptosis;
- To inhibit angiogenesis.

References
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