WHY GENE THERAPY FOR ARTHRITIS

Although arthritis is not necessarily a genetic disease, it and other autoimmune diseases are good candidates for treatment by gene therapy. Arthritis, including rheumatoid arthritis, is currently incurable with joint replacement being the final recourse for many patients (Praemer et al., 1992). One difficulty with treating arthritis is targeting of therapeutic agents to the joint, the site where the disease is predominantly manifest. Steroids have proven to be effective at high doses, but the associated side effects make prolonged steroid treatments impossible. Certain proteins such as interleukin-1 receptor antagonist protein, are also therapeutic in animal models of arthritis (Smith et al., 1991), but only high, sustained, systemic doses will effectively treat the joint. With existing delivery systems, the required levels of proteins can only be obtained by injection. Therefore, we have proposed that high intra-articular concentrations of therapeutic proteins could be achieved through delivery of genes encoding the therapeutic proteins to the synovial lining of the joint (Bandara et al., 1992, 1993; Evans and Robbins 1994b,c; Evans et al., 1993). The large surface area of the synovium, the ability of the synovium to sequester intra-articularly injected materials, and the ease by which joints can be injected make the joint an attractive target for gene delivery (Hun and Evans, 1994). We have demonstrated the ability to efficiently transfer and express genes intra-articularly (Bandara et al., 1993; Hung et al, 1994a). Moreover, our results clearly show that intra-articular expression of therapeutic gene products is highly effective in halting the pathophysiological changes associated with antigen-induced arthritis in the rabbit. The results of our initial experiments demonstrate the feasibility of treating arthritis by gene therapy and have led to approval of the first human gene therapy trial for arthritis.

METHODS FOR SYNOVIAL GENE TRANSFER

Two approaches can be taken for transferring therapeutic genes to the joint. One involves direct, in situ, gene transfer. This can be achieved by direct injection of a vector, viral or nonviral, into the joint space where it can come in direct contact with the synovial lining. The second approach is an ex vivo, indirect method where the synovial cells are isolated, propagated in culture, genetically modified using viral or nonviral vectors and then transplanted back into the rabbit knee by intra-articular injection (Hung et al., 1994b).

The vectors that can be used for gene transfer to synovial cells are shown in Table 1. Retroviruses are able to stably infect synovial cells in culture. The requirement for cell division for retroviral infection prevents infection of quiescent synovial cells after direct injection into the joint. However, in an inflamed joint with associated cell proliferation, successful retroviral gene transfer has been observed (unpublished observations). Adenovirus and herpes simplex virus vectors also are able to efficiently infect synovial cells in culture and in vivo. However, the expression of the transgene is transient. In addition, the current adenovirus and herpes virus vectors are not completely defective and still express low levels of viral proteins. An immune response against the viral antigens, especially in the inflamed joint, could be induced by infected cells. Adeno-associated virus (AAV), a parvovirus, is able to infect synovial cells in culture and, in theory, synovial cells in vivo. The difficulty
in generating high titer recombinant AAV virus has made assessment of its ability to infect synovium in vivo difficult.

For nonviral delivery systems, several different methods exist for delivery of therapeutic genes to synovial cells in vivo and ex vivo. Of these methods, cationic liposomes (Gao and Huang, 1991) have shown the most promise in preliminary experiments since certain cationic liposomes can readily transfect primary cultures of synovial cells. So far, attempts using cationic liposomes for in vivo gene delivery have met with only marginal success. Naked DNA also can be taken up in vivo at a low frequency, most likely by the type A, macrophage-like synovial cell. In theory, DNA conjugates also should be able to mediate transfection of synovial cells in vivo.

TREATMENT OF ARTHRITIS BY GENE TRANSFER IN ANIMAL MODELS

We have been developing both in vivo and ex vivo methods for gene delivery to synovial cells. Most progress to date has been made with the ex vivo method. Synovial cells can be readily isolated from the rabbit knee by partial synovectomy and propagated in culture (Hung et al., 1994b). These cells can be readily infected with an amphotropic retroviral vector. For our initial experiments the gene encoding interleukin-1 receptor antagonist protein (IL-1ra or IRAP) was used (Arend, 1991; Evans and Robbins, 1994a). IL-1ra competes with IL-1 for binding to the IL-1 type 1 receptor, but does not itself have agonist activity. IL-1 has been implicated as a key mediator of inflammatory arthritis in animal models, particularly in the rabbit knee. Intra-articular injection of recombinant IL-1 induces a leukocytic cellular infiltrate, synovial cell hyperplasia, cartilage breakdown, and inhibition of matrix synthesis by cartilage. Using a retroviral vector expressing IL-1ra (MFG-IRAP), synovial cells in culture can be genetically modified to express 500 nanograms IL-1ra per 106 cells every 48 hours. These genetically modified cells were then injected into the knee to induce an immune response with associated pathologies not unlike those found in human rheumatoid arthritis. Synovial cells, genetically modified to express IRAP, were then injected into the knee the following day and the knee was analyzed at different points in time. The intra-articular expression of IRAP dramatically reduced all pathophysiological parameters including cell infiltration, synovial thickening, cartilage breakdown, and inhibition of cartilage proteoglycan synthesis. These results demonstrate the feasibility and efficacy of treating arthritis by gene transfer.

IL-1ra also has been delivered to the joint and expressed using direct injection of recombinant adeno virus vectors (B. Roessler, University of Michigan, Ann Arbor) personal communication). The initial level of expression of IL-1ra was significantly higher than that obtained using transplantation of genetically modified synoviocytes. However, expression of IL-1ra dropped significantly after the first week. Defective herpes virus vectors also are able to efficiently infect the synovial lining after intra-articular injection (unpublished observations). The ability of adeno virus and herpes simplex virus vectors to efficiently deliver and express genes intra-articularly suggests that these viral vectors also could be effective in treating diseases of the joints by gene transfer. The development of better adenoviral and herpes virus vectors that are less immunogenic, less toxic, and express longer might allow for their clinical application to the treatment of arthritis.

Clinical Protocol for Gene Therapy of Arthritis

The result obtained with the rabbit knee have led to submission and approval of a clinical protocol to test the safety and efficacy of treating rheumatoid arthritis by gene therapy. The protocol involves the infection of human synovial cells with a retroviral vector carrying IRAP into the metacarpal phalangeal (MCP) joints of patients scheduled to undergo MCP joint replacement. The synovial cells to be used for infection will be isolated from another joint, such as the wrist, which is scheduled for arthroplasty prior to MCP joint replacement. One week after the transplant of the modified synovial cells, the joint will be removed and analyzed for IRAP expression and for pathophysiological changes. In this manner the feasibility of treating arthritis by gene transfer can be ascertained without the patients having to undergo any additional surgical procedures. It is important to note that the clinical trial is not designed to treat arthritis. The joints into which genetically modified cells will be injected are at the terminal
stage of disease. Instead, the trial is designed to demonstrate successful gene transfer and expression as well as the safety of the procedure. The efficacy of the treatment will be determined in subsequent trials using gene transfer to joints at an earlier stage of disease.

Systemic Gene Therapy for Arthritis

The local delivery of therapeutic proteins can be used to halt or reverse the pathophysiological changes in the diseased joint. However, rheumatoid arthritis is a systemic disease, affecting multiple joints. Thus the systemic expression of a therapeutic protein, or several proteins, could be more effective in treating the disease. In this regard, intravenous delivery of an antibody against TNF-α has shown improvement in both mouse models of arthritis as well as in phase I/II clinical trials (Elliot et al., 1993). We are examining the effect of systemic expression of potentially therapeutic genes following infection of mouse hematopoietic stem cells and reconstitution of lethally irradiated recipients. In this manner, high systemic levels of IL-1ra and soluble TNF-α receptor (p75) have been achieved. These mice are currently being examined for inhibitory effects on both the initiation and progression of antigen-induced arthritis. For the clinical application of gene therapy to the treatment of systemic diseases, a variety of different delivery systems could be used. CD34+ stem cells from the peripheral blood represent an attractive target for retroviral-mediated gene delivery. Alternatively, differentiated hematopoietic cells, such as T cells, could be used for gene delivery. Myoblast and fibroblast cell implants also have been used as gene delivery vehicles. Even direct injection of DNA or viral vectors into muscle has been shown to lead to systemic levels of gene expression. Thus it is possible that gene therapy could be applied to the systemic treatment of autoimmune diseases such as arthritis.

Therapeutic Proteins

The initial experiments towards developing a gene therapy for arthritis have used genes encoding cytokine inhibitors such as IL-1ra or soluble TNF-alpha receptor. Similarly, soluble receptors of IL-1 also are being tested. However, other proteins may also be therapeutic for treating arthritis (see Table 2). Cytokines IL-4, IL-10, and, in particular, the viral form of IL-10 encoded by Epstein-Barr virus may be anti-inflammatory and immunomodulatory. The expression of metalloproteinase inhibitors such as TIMP-1 and TIMP-2 may have protective effects on the cartilage. Anti-adhesion molecules such as soluble ICAM-1 or soluble CD44 may inhibit cell-to-cell contact and cell matrix interactions, whereas soluble CD28 may block T-cell activation. Free radical antagonists such as superoxide dismutase and NO blockers could prove therapeutic. Finally, the expression of cartilage growth factors such as IGF-1 and TGF-β1 could promote cartilage repair. It is likely that specific combinations of these potentially therapeutic proteins will need to be employed to halt the progression of the disease and to promote repair of the joint.

ANIMAL MODELS

In addition to treating arthritis and other inflammatory and autoimmune diseases, gene transfer technology can be used to evaluate the role of specific proteins in the initiation and progression of the disease. We have successfully expressed high levels of IL-1β in the joint by gene transfer, resulting in dramatic induction of cellular infiltrate and destruction of the joint. The effects of chronic IL-1β expression can now be evaluated using this gene transfer technology. Similarly, genes encoding proteins thought to be involved in progression of the disease, such as metalloproteinases, could be expressed intra-articularly and their effects evaluated. In addition to understanding the role of specific proteins in the disease process, animals expressing specific proteins intra-articularly could be used to test specific anti-arthritic drugs.

FUTURE DIRECTIONS

The initial experiments performed by our laboratory and by others have demonstrated the feasibility of gene transfer to

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<thead>
<tr>
<th>Protein</th>
<th>Rationale</th>
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<tr>
<td>IRAP/IL-1ra, IL-1 soluble receptors type I and type II</td>
<td>Antagonize IL-1, anti-inflammatory, chondroprotective</td>
</tr>
<tr>
<td>TNF-α soluble receptors p55 and p75</td>
<td>Antagonize TNF-α, anti-inflammatory, chondroprotective</td>
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<tr>
<td>IL-4</td>
<td>Anti-inflammatory, induces IL-1ra</td>
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<tr>
<td>IL-10 (vIL-10)</td>
<td>Anti-inflammatory, induces IL-1ra</td>
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<tr>
<td>TGF-β</td>
<td>Antagonizes responses to IL-1, promotes cartilage matrix synthesis</td>
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<tr>
<td>TIMPs</td>
<td>Inhibit metalloproteinases</td>
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<tr>
<td>Superoxide dismutase</td>
<td>Antagonizes oxygen-derived free radicals</td>
</tr>
<tr>
<td>Soluble adhesion molecules</td>
<td>Block cell-cell, cell-matrix interactions</td>
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<tr>
<td>IGF-1, bFGF</td>
<td>Promote cartilage matrix synthesis</td>
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the synovium using both in vivo and ex vivo techniques. Our ex vivo experiments demonstrating therapeutic efficacy in an antigen-induced model of arthritis in the rabbit have led to the approval of the first human gene therapy trials for a non-lethal disease. These initial and subsequent clinical trials should determine whether gene therapy can reverse the pathophysiology associated with arthritis. However, if gene therapy is to become an accepted method for treating inflammatory diseases such as arthritis, an easy, efficient, and safe method for gene delivery to synovium will be required. For example, a lyophilized therapeutic DNA complex, which could be mixed with saline and intra-articularly injected during an outpatient procedure, would allow for the widespread application of gene therapy for diseases of the joint. The initial gene therapy experiments for arthritis have now paved the road for clinical gene therapy applications to spread from the arena of lethal cancers and genetic diseases to the arena of non-lethal, non-classical genetic diseases.

REFERENCES

Gene Therapy for Inherited Neurological Disease
Rosanne M. Taylor

INTRODUCTION
Most inherited neurological disorders are not amenable to existing treatments, however they are potential candidates for gene therapy, an approach that combines recent developments in molecular diagnosis with novel gene transfer techniques. Genes may be transferred directly to neural tissue using viral vectors or by physical methods, or indirectly by injection of cell vehicles that express therapeutic genes. The application of gene therapy to the treatment of neurological disease is at an early stage, and inherent problems, such as accessing sites of injury or degeneration and stimulating neuronal regeneration, have yet to be resolved. Promising gene transfer techniques have many potential clinical applications for brain diseases, so it is imperative that they be validated prior to use in humans. For this purpose, there are many suitable animal models of neurological disease that can be used to provide a scientific rationale for safe and effective treatment. This review focuses on inherited neurological disorders and gene therapy approaches to their treatment.

CHALLENGES TO THE TREATMENT OF NEUROLOGICAL DISEASE
Compared to other organs, the brain is particularly vulnerable to degenerative disease, and it is a difficult organ to...