Introduction

Arthritic diseases are common crippling conditions for which there are no cures and few useful treatments. Nevertheless, recent research into the biology of rheumatoid arthritis (RA) and osteoarthritis (OA), the two most common forms of arthritis, has identified a number of potentially anti-arthritic proteins with promising anti-inflammatory, immunomodulatory or chondroprotective properties. A number of these proteins have been evaluated in animal models of arthritis, with encouraging results.

Clinical application of such mediators is greatly hindered by the lack of a suitable means for delivering them to patients with arthritis. Oral administration of proteins is ineffective, and delivery by injection is inefficient because of the rapidity with which they are cleared. To circumvent these limitations, we have suggested that instead of delivering the therapeutic proteins themselves, it would be better to deliver the genes that encode them. In this sense, we are proposing gene transfer as a biological drug-delivery system for secreted anti-arthritic proteins [1–4]. Although this concept can be extended to include the delivery of other gene products, such as anti-sense RNA or transcription factors, which act intracellularly, the use of genes encoding secreted proteins stands the greatest chance of early success as it can be used in an ex vivo fashion and does not require unreasonably high transduction efficiencies. In this context, the most immediate questions to be answered are: which genes are anti-arthritic? Where should they be transferred? How should they be transferred?

Which genes are anti-arthritic?

Most data are available for RA, which is a chronic erosive inflammatory condition with important systemic components; it is thought to be autoimmune in nature. Comprehensive treatment of RA requires that both the loss of cartilage and the inflammation within joints be arrested, and that the systemic components of the disease are brought under control.

One therapeutic possibility is to target the co-stimulatory pathways, such as B7/CD28 and CD40/CD40L, which are required for lymphocyte activation [5]. Such intervention has the potential not only to suppress unwanted immune reactivity, but also to induce tolerance. The use of soluble ligands and receptors is an attractive approach to achieving this.

Alternative targets are the cytokines that drive many of the intra-articular pathophysiological events [6]. Interleukin (IL)-1 and tumour necrosis factor α (TNF-α) have emerged as key mediators in this respect [7]. Naturally occurring antagonists of these cytokines exist. Soluble forms of both IL-1 receptors and both TNF-α receptors occur at sites of inflammation, whereas IL-1Ra acts as a receptor antagonist [7]. These antagonists and neutralizing antibodies to TNF-α show anti-arthritic activity in animal models of arthritis, and several have entered human trials [8–10]. These trials have illustrated the problems associated with the sustained delivery of therapeutic proteins to patients with chronic conditions. Intravenous loading of the patients was followed several weeks later by a flare [8], and daily subcutaneous self-injection was...
Arthritis, as its name suggests, is a disease that affects the joints. This being so, it makes sense to transfer anti-arthritic genes to the affected joints, so that the highest concentrations of the gene products occur in direct contact with the joint space, it is an obvious target for local gene delivery to joints. Certain applications in OA, however, may require gene transfer to chondrocytes.

**Where to transfer anti-arthritic genes?**
Arthritis, as its name suggests, is a disease that produces its main pathological effects within joints. This being so, it makes sense to transfer anti-arthritic genes to the affected joints, so that the highest concentrations of the gene products occur intra-articularly. As well as solving the drug-delivery problems described above, intra-articular delivery ensures that extra-articular tissues receive reduced exposure to the gene products. This brings the important advantage of minimizing side effects, a complication for which existing anti-arthritic drugs are notorious. Because the synovium has a large surface and is in direct contact with the joint space, it is an obvious target for local gene delivery to joints. Certain applications in OA, however, may require gene transfer to chondrocytes.

Local intra-articular gene delivery is well suited to conditions such as OA, where few joints are affected and there are no major systemic disturbances. However, because RA is a polyarticular systemic disease, we have also investigated the possibility of introducing anti-arthritic genes into locations where the secreted gene products gain access to the systemic circulation. Experiments in which such genes are transferred to the haemopoietic stem cells of mice have confirmed that systemic delivery of this nature can result in high circulating levels of biologically active products. Additional potential targets include muscle, lymphocytes and artificial organoids.

**Methods of gene transfer in arthritis**
As with other areas of gene therapy, investigators have available a variety of viral and non-viral vectors, and may use them according to *in vivo* or *ex vivo* strategies. Screening a variety of vectors for their ability to transfer the lacZ gene into the synovial lining of the rabbit knee joint showed quite clearly that *ex vivo* gene delivery could be accomplished with retroviral vectors, and that *in vivo* gene delivery was best accomplished with adenoviral vectors. However, the first generation (E1A−) adenoviral vectors used in this study provoked a considerable degree of synovial inflammation. Inflammation was also noted after injection of similar adenoviral vectors into the knee joints of mice and guinea pigs. Roesler et al. [24], however, did not observe this effect in the knee joints of rabbits.

For systemic delivery, *ex vivo* gene transfer using retroviruses has been successful for delivery to a number of extra-articular tissues. Direct injection into the bloodstream or accessible solid organs such as muscle should also be possible once problems of immunogenicity and long-term expression have been overcome.

**Other issues**
Arthritic diseases are chronic and usually follow an unpredictable course of flares and remissions. Any gene treatments should thus aim to achieve prolonged regulated gene expression or be so minimally invasive as to permit frequent repeat dosing. None of these desiderata have yet been met, but the volume of research activity surrounding these issues is such as to permit optimism about future progress.

**Preclinical data**
Local delivery of cDNAs encoding a number of potentially anti-arthritic genes has been achieved
Gene Therapy

by \textit{ex vivo} transfer with a retrovirus and \textit{in vivo} transfer with adenovirus (Table 1). In each case there was evidence of gene expression and an anti-arthritic effect. The strong agreement between the data of four different groups of investigators working independently at different centres using different vectors and different animal models of RA permits considerable optimism about the potential of gene therapy to treat this disease.

The first experimental study investigating the use of gene therapy to treat OA has just been completed. This utilized a dog model, in which transection of the anterior cruciate ligament in one knee joint leads to osteoarthritic changes. \textit{Ex vivo} transfer of the human IL-1Ra cDNA to the synovial fibroblasts isolated from the lining of the joint 2 days after transection of the ligament protected the articular cartilage from the early degenerative changes that otherwise follow this procedure [32]. OA may also be treated by the transfer of genes to cartilage. The \textit{ex vivo} transfer of a marker gene to chondrocytes using a retrovirus has just been reported [33].

In the context of systemic gene delivery for RA, potentially anti-arthritic genes have been transferred to the skeletal muscle [20], haemopoietic stem cells [18,19] and lymphocytes [21] of mice. Raz et al. [20] injected plasmid DNA encoding IL-1, IL-4 or transforming growth factor $\beta$ into murine muscle and were able to increase or decrease the immune responses of the animals depending on the cytokine whose cDNA was transferred. This result is quite remarkable in view of the low levels of gene expression that such procedures generally produce.

Transfer of IL-1Ra and TNF-\textit{z}sRII genes into haemopoietic bone marrow cells in mice led to life-long transgene expression and high sustained serum concentrations of the gene product [18,19]. Certain responses to endotoxin challenge were attenuated in these mice [19].

Retroviral transfer of the TNF-\textit{z}sRII gene to splenocytes of mice with collagen-induced arthritis was able to inhibit the development of the arthritis that otherwise follows the transfer of these cells to recipients with severe combined immunodeficiency (SCID) [21]. Lymphocytes are an attractive target because they infiltrate rheumatoid synovia, and some subsets may home to joints.

The only preclinical evidence that gene therapy may be effective in human arthritic tissues comes from the results of experiments in which human synovial fibroblasts and human cartilage were co-implanted into SCID mice [34]. Under these conditions, rheumatoid synoviocytes degrade the human cartilage by both direct invasion and stimulating chondrocytic chondrolysis. Prior retroviral transduction of the synoviocytes with the human IL-1Ra gene inhibited the latter process. In these experiments, transgene expression was still evident 60 days after implantation of the cells. This suggests that the expression of human IL-1Ra in rabbit knees is limited to approximately half this value by immune mechanisms.

\textbf{The first human trial}

These promising preclinical findings led to the first human trial of gene therapy for arthritis [35]. A human IL-1Ra cDNA was selected as the gene of choice as it had shown promise in animal models of RA.

\begin{table}[h]
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Gene & Species & Method & Arthritis model & Effect on disease & Reference \\
\hline
IL-1Ra & Rabbit & \textit{Ex vivo} & IL-1 & Inhibitory & [25,26] \\
& & & Antigen-induced arthritis & Inhibitory & [27] \\
& Rabbit & \textit{In vivo} & IL-1 & Inhibitory & [28] \\
Rat & \textit{Ex vivo} & Streptococcal cell wall & Inhibitory & [29] \\
Mouse & \textit{Ex vivo} & Collagen-induced arthritis & Inhibitory & [30] \\
IL-1sRI & Rabbit & \textit{In vivo} & Antigen-induced arthritis & Inhibitory & [31] \\
TNF-\textit{z}sRII & Rabbit & \textit{In vivo} & Antigen-induced arthritis & Little effect alone, but potentiated the effects of IL-1sRI & [31] \\
\hline
\end{tabular}
\caption{Transfer of anti-arthritic genes to joints: effects in animal models of RA}
\end{table}
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models and because IL-1Ra protein has such a favourable toxicological profile. Moreover IL-1Ra protein had already been safely administered to normal human volunteers and to patients with inflammatory conditions. An ex vivo delivery system was selected because of its promise in preclinical testing and because with ex vivo methods no infectious agents are introduced directly into the patient. All genetic manipulations occur outside the body, and the transduced cells can be extensively tested for safety before reimplantation. As an additional safety measure, the genes are introduced into joints 1 week before their surgical removal for the implantation of artificial joints. This has the additional advantage of providing joint tissue for further analysis.

To eliminate the possibility of germline transmission of the new genetic information, this trial is limited to postmenopausal women. Other inclusion criteria are a diagnosis of RA and the need for joint replacement surgery on the metacarpophalangeal joints numbers 2–4 (knuckles) of one hand and an additional surgical procedure on at least one other joint. The latter provides the opportunity to recover autologous tissue, from which synovial fibroblasts are propagated. Half of the cells are retrovirally transduced with the IL-1Ra cDNA and the other half are not. Having confirmed that the transduced cells produce sufficient IL-1Ra, they and the untransduced cells are subjected to detailed safety testing before being injected into the patient’s knuckle joints. Two of the knuckles receive untransduced cells, and the other two receive transduced cells. After 1 week the joints are surgically removed and the tissues examined for evidence of successful gene transfer, gene expression and a local biological response to the IL-1Ra produced from the transgene. Because of the advanced nature of the disease and the short dwell time of the gene, no clinical improvement is expected, although patients are closely monitored clinically as part of the protocol.

In January 1996, the U.S. Federal Drug Administration gave permission for nine patients to be treated in this manner. So far two have been enrolled and one treated. The procedure was extremely well tolerated by the patient and no adverse effects have been noted. Tissue is in the process of being analysed for evidence of successful gene transfer and expression.

Progress to date has been rapid and permits optimism that gene therapy may become a treatment option for a variety of autoimmune [36], orthopaedic [37] and rheumatological conditions [4].

This work was supported, in part, by NIH grants PO1-DK-44935, RO1-AR-43623 and AR-08391. We thank Mrs. Duerring for kindly typing the manuscript.

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Since the first successful HLA-matched sibling bone marrow transplants were performed in two distinct primary immunodeficiency disorders over 30 years ago, transplantation technology has developed considerably, and the cure rate for severe combined immunodeficiency (SCID) using matched sibling donors is now over 90%. However, for only 30% of patients does such a donor exist, and for haplo-identical parental grafts, success rate falls to 50% [1]. Transplantation for immunodeficiencies other than SCID, in which the presence of competent host lymphocytes can lead to graft rejection, is complicated by the need for additional immunosuppression. Under these circumstances, mortality after a fully matched sibling transplantation is currently around 30%. Complications relate to the degree of immunosuppression required to achieve engraftment, and to graft versus host diseases (GvHD), which is responsible for up to 25% of post-transplant mortality. Prevention of GvHD by T-cell depletion of donor cells is effective, but is associated with increased rates of graft rejection.

Rapid progress in understanding the molecular basis for some of these disorders, including adenosine deaminase (ADA) deficiency, chronic