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Gene therapy with the interleukin-1 receptor antagonist for the treatment of arthritis

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Local gene therapy is a promising approach for the treatment of arthritis. The first clinical trial of this strategy involved nine postmenopausal women requiring unilateral sialistic implant arthroplasty of metacarpophalangeal (MCP) joints 2–5 for the treatment of rheumatoid arthritis (RA). Autologous synoviocytes were recovered and transduced with a retrovirus, MFG-IRAP, which carries human interleukin-1 receptor antagonist (IL-1Ra) cDNA. In a dose-escalation, double-blind fashion, two MCP joints on each subject's hand were injected with genetically modified cells, and the other two MCPs were injected with unmodified cells. After 1 week, the injected joints underwent replacement arthroplasty. Examination of retrieved synovia confirmed successful gene transfer and intra-articular transgene expression. A subsequent, similar study involving two subjects with RA provided clear evidence of a marked clinical response to the gene therapy. No adverse events occurred during either study. These highly promising findings encourage further development of genetic treatments for the arthritides.

Keywords: adeno-associated virus, arthritis, clinical trials, gene therapy, interleukin-1 receptor antagonist, retrovirus, synoviocyte

Although neither rheumatoid arthritis (RA) nor osteoarthritis (OA) are Mendelian disorders they, like other arthritides, are amenable to treatment with gene therapy [1]. The underlying concept is to use gene transfer as a method for delivering anti-arthritic gene products in a safe, targeted, sustained and cost-effective manner that provides a superior clinical outcome. Although there are several possible strategies for achieving this [1], most progress has been made with the local, intra-articular delivery of genes to the synovial linings of individual diseased joints (Figure 1) [2]. There is a wealth of preclinical data confirming the efficacy of local, intra-articular gene therapy in experimental models of RA [3]. A small number of studies also demonstrate efficacy in animal models of OA [4].

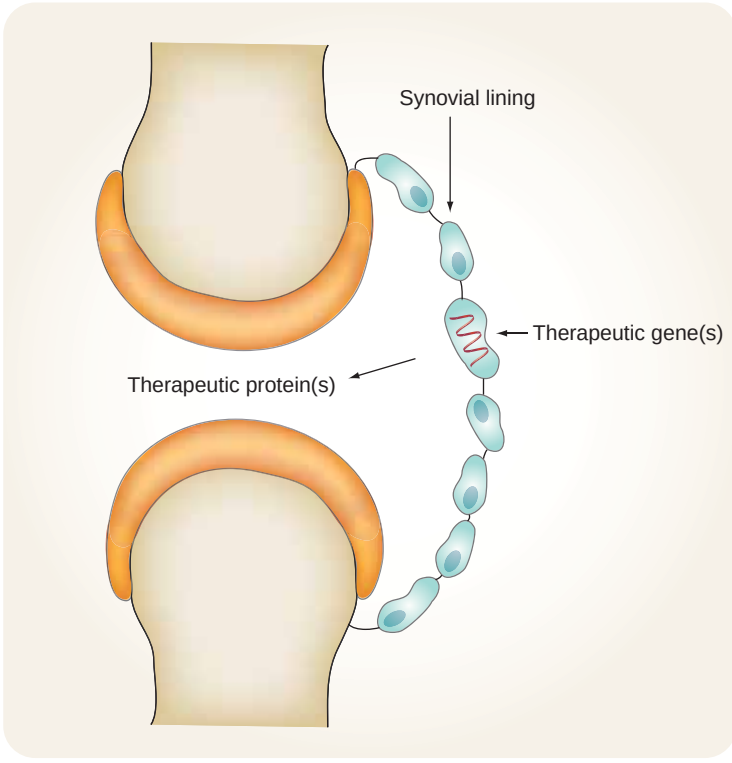
As described in detail elsewhere [1], genes may be delivered to synovia by *ex vivo* or *in vivo* protocols involving several different vectors and a variety of different genes. We have selected the interleukin-1 receptor antagonist (IL-1Ra) cDNA for translation into human clinical trials [5]. There are several reasons for this. First, IL-1 is likely to be a key mediator of disease in both RA and OA, where it helps mediate the inflammatory and erosive components of joint dysfunction. Second, IL-1Ra is a naturally occurring antagonist of IL-1 that, in its recombinant form, has already been widely used at high doses in both healthy individuals and those suffering from disorders, such as RA, septic shock and graft-versus-host disease [5]. The data confirm that recombinant IL-1Ra protein is safe, even at enormously high doses. This is a key issue for gene therapy, where safety is always of major concern, especially for non-terminal diseases such as arthritis. Moreover, the dose–response curve of IL-1Ra shows an uncomplicated rectangular hyperbola with no evidence of pleiotropism. Thus, beyond expressing sufficient IL-1Ra to block the intra-articular effects of IL-1, the level of transgene expression need not be finely regulated. Finally, data from clinical trials of recombinant IL-1Ra (Kineret®) in RA suggest that efficacy is severely compromised by its short biological half-life and the consequent inability to deliver a sustained, therapeutic concentration of the protein to joints. This is precisely the sort of limitation that gene transfer can overcome.

Based upon the best available gene transfer technology at the time, we developed an *ex vivo* protocol in which a retrovirus was used to transfer human IL-1Ra cDNA to autologous synovial fibroblasts, which were subsequently injected into target joints [6].

Preclinical data

A derivative of the Moloney murine leukemia virus (MFG) was engineered as the vector. All viral coding sequences have been deleted from this vector, which retains essential *cis*-acting elements, including the 3′ and 5′ long terminal repeats, the packaging sequence ψ and the splice donor and acceptor sites. The transgene, a cDNA containing the entire coding region of

Figure 1. Gene delivery to the synovium for local, intra-articular therapy.



Vectors are used to transfer cDNAs whose products have anti-arthritic properties. In many cases these will be secreted proteins, as indicated here. The products are synthesized endogenously in a sustained fashion, with minimal exposure of extra-articular tissues.
Adapted with permission from Bandara G, Robbins PD, Georgescu HI, Mueller GM, Glorioso JC, Evans CH: Gene transfer to synoviocytes: prospects for gene treatment for arthritis. *DNA Cell Biol.* 11, 227–231 (1992).

human IL-1Ra, was inserted in place of the viral *env* gene, retaining the position of the native initiation codon (Figure 2). This led to the production of high levels of authentically spliced mRNA, with transcription driven by the viral 5´ long terminal repeat and polyadenylation signals provided by the 3´ long terminal repeat. High translation efficiency results in the production of large amounts of authentically processed human IL-1Ra by cells infected with this vector, known as MFG-IRAP (IRAP was an alternative acronym for the IL-1Ra at the time).

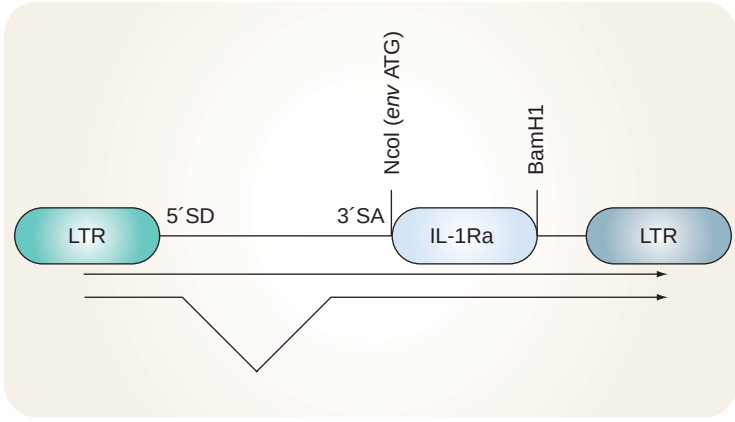
The rabbit knee was used as the model system [7]. This is large enough to permit the surgical synovectomy necessary for recovering autologous synovium and for reliable intra-articular injection, but small enough to be economical and convenient. Moreover, the rabbit knee joint is approximately the same size as the human metacarpophalangeal (MCP) joints that would be injected in the subsequent human trials. Monolayers of autologous synovial fibroblasts were established, transduced with MFG-IRAP and returned to the knee joints of the donor rabbits. After the injection of 10^6–10^7 genetically modified, autologous synovial fibroblasts, up to 20 ng human (h) IL-1Ra could be recovered from the knees in 1 ml of lavage fluid [7]. Of considerable relevance to the inflammatory arthritides, hIL-1Ra expression was higher in inflamed than in normal joints [8]. hIL-1Ra expression persisted within the knee joint at a declining rate for up to 6 weeks in some animals [7]; however, transgene expression was extinguished more typically within 3–4 weeks. It is now known that extinction of transgene expression reflects an immune reaction by the rabbit to the human protein, and there is evidence to suggest that long-term transgene expression is possible when autologous genes are transferred [9].

Using this system, it was possible to block biological responses to intra-articular challenge with IL-1 [7,10] and to treat antigen-induced arthritis in the rabbit knee [8]. Interestingly, IL-1Ra gene therapy proved particularly effective in protecting the articular cartilage. Subsequent investigators confirmed that *ex vivo*, retrovirally mediated, intra-articular transfer of hIL-1Ra cDNA suppressed streptococcal cell-wall arthritis in rats [11] and zymosan- and collagen-induced arthritis in mice [12]. Thus, the combined experience of three independent laboratories confirmed the efficacy of this specific therapy in four different models of RA using three different species of laboratory animals.

Confidence that the technology would be effective *in vivo* in humans was provided by experiments in which human synovial fibroblasts were transduced with MFG-IRAP and coimplanted into severe combined immune deficient (SCID) mice with fragments of human cartilage [13]. In the presence of IL-1Ra cDNA, but not a marker gene, there was a strong inhibition of chondrocytic chondrolysis.

Safety studies included the introduction of high doses of synovial fibroblasts transduced with MFG-IRAP into rabbits by intra-articular and intravenous injection [6]. Tracking studies confirmed that the genetically modified cells rarely escaped from the joints into which they had been injected, and even high doses of these cells caused no pathology when injected into the blood stream. We also transduced the

Figure 2. Key elements of the MFG-IRAP genome.



All coding sequences have been removed from the Moloney murine leukemia virus, retaining the 5´ and 3´ long LTRs, the packaging sequence ψ and the SD and SA sites. The entire coding sequence of the human IL-1Ra cDNA is inserted into the former site of the viral *env* gene, retaining the original ATG initiation codon.
IL-1Ra: Interleukin-1 receptor antagonist; LTR: Long terminal repeat; SA: Splice acceptor; SD: Splice donor.

hematopoietic stem cells of mice with MFG-IRAP, leading to a high, life-long, systemic expression of hIL-1Ra without producing noticeable pathology [14].

Human protocol

Based upon the *ex vivo* delivery method developed in the rabbit model, a Phase I human protocol was constructed targeting the MCP joints of subjects with advanced RA (Figure 3) [6]. As this was the first protocol to come before the regulatory bodies for the gene therapy of a nonlethal disease, considerable attention was devoted to the issue of safety. Although the preclinical data gave no indication of any major safety concerns, the safety margin was increased by two features of the human protocol. First, the issue of possible germ-line transmission was dealt with by gene delivery to postmenopausal females. Second, the inclusion criteria specified subjects who required unilateral sialistic implant arthroplasty of MCP joints 2–5. This provided the opportunity to introduce genes into MCP joints that would be surgically removed 1 week later. As the preclinical data suggested that the injected cells did not leave the joints into which they were injected, they would be removed by arthroplasty. An additional advantage of this step was the ability to recover large amounts of tissue for laboratory analysis. A time period of 1 week was chosen as a length of time that was sufficient to address the feasibility of the procedure and give preliminary indications concerning safety, while being short enough to minimize the chances of unforeseen adverse events.

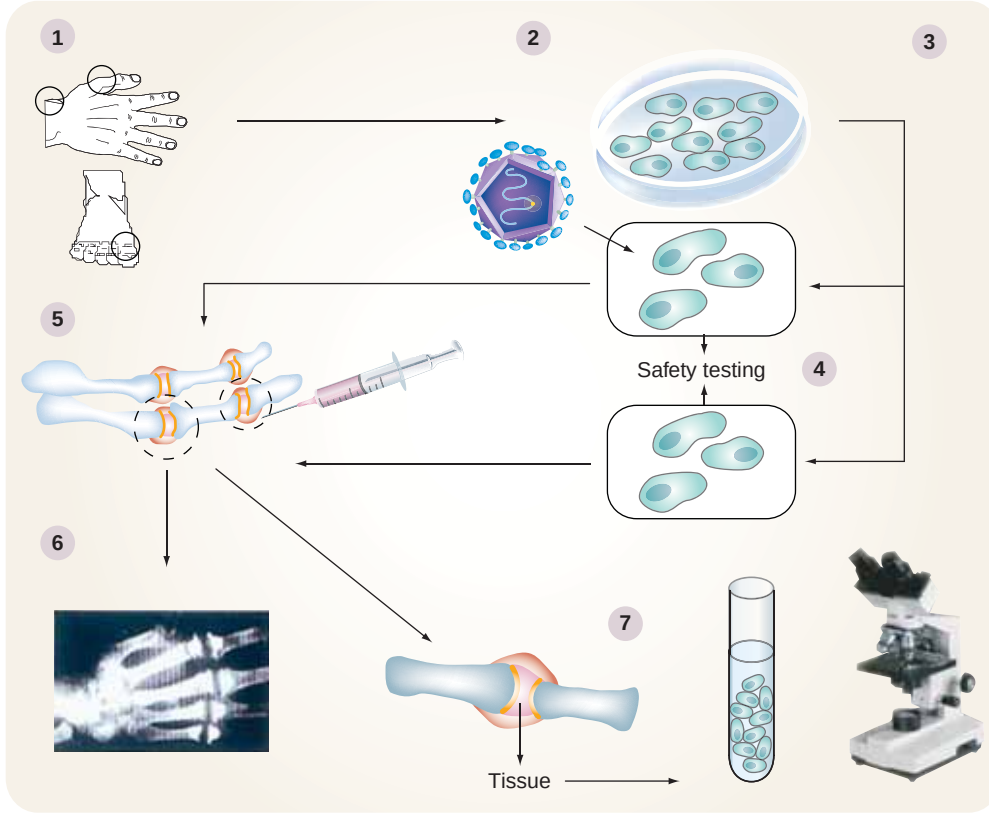
Autologous synovium was recovered at the time of prior arthroplasty required for the surgical management of the disease in joints other than the target MCPs. Cultures of synovial fibroblasts were established and half of the cultures were transduced with MFG-IRAP. Nine subjects underwent gene transfer, divided into three groups of three subjects receiving low (10^6), medium ($1.5–5 \times 10^6$) and high ($6.5 \times 10^6–10^7$) doses of cells per MCP joint. In a double-blind fashion, two MCP joints on each hand were injected with unmodified cells and the other two MCP joints received genetically modified cells. After 1 week, all injected joints were removed and examined for transgene expression at the RNA and protein levels by reverse transcriptase polymerase chain reaction, *in situ* hybridization, immunohistochemistry and enzyme-linked immunosorbent assay (Figure 3).

Once this trial was underway at the University of Pittsburgh Medical Center, PA, USA, a second protocol was initiated at the University of Düsseldorf, Germany. This employed the same basic protocol but the German study allowed for subsequent synovectomy of the injected joints, rather than total joint replacement, and a period of 1 month between the injection and removal of the genetically modified cells. Eliminating the need for total joint replacement surgery enabled the recruitment of younger patients with more active disease who were more likely to show a clinical response to the gene therapy. The dwell time of 1 month for the transferred genes also increased the likelihood of a clinical response. Due to anecdotal reports of symptomatic improvement in subjects participating in the US study, the German protocol included the assessment of pain and measurement of swelling in MCP joint 1. Permission was granted for six subjects to be treated in Düsseldorf, but the development of leukemia in children receiving gene therapy for X-linked SCID in an unrelated study in France curtailed the trial after only two subjects had completed the protocol.

Clinical findings

All nine subjects in the US study successfully completed the protocol and there were no adverse events related to the study [15]. The data

Figure 3. Gene transfer to human rheumatoid joints.



1. Surgery of the hand or foot provided autologous synovium. **2.** These were used to establish cultures of synovial fibroblasts. **3.** Half of these were transduced with MFG-IRAP, while the other half remained as untransduced controls (**4**). **5.** After safety testing, two metacarpophalangeal (MCP) joints on one hand were injected with control cells and the other two were injected with transduced cells. **6.** After 1 week, all four MCP joints underwent total joint replacement surgery and the retrieved tissues were analyzed for evidence of successful engraftment and intra-articular transgene expression by the injected cells (**7**).
Adapted with permission from the University of Pittsburgh, PA, USA. Evans CH, Ghivizzani SC, Kang R *et al.*: Gene therapy for rheumatic diseases. *Arthritis Rheum.* 42(1), 1–16 (1999).

clearly confirmed that, as in laboratory animals, the genetically modified cells adhered to the synovial linings of the recipient joints, where they continued to express the transgene (Figure 4). IL-1Ra expression was enhanced in 11 out of 12 joints receiving the highest doses of the two genetically modified, autologous synovial fibroblasts. Several subjects reported a symptomatic improvement in one or more joints, but this was ascribed to the placebo effect commonly seen in arthritis efficacy trials.

Both subjects in the German study reported large improvements in pain scores and reduced swelling in joints receiving the transgene, but not in placebo joints [Unpublished Data]. Improvement began soon after injection of the autologous synoviocytes and persisted for the month of the study.

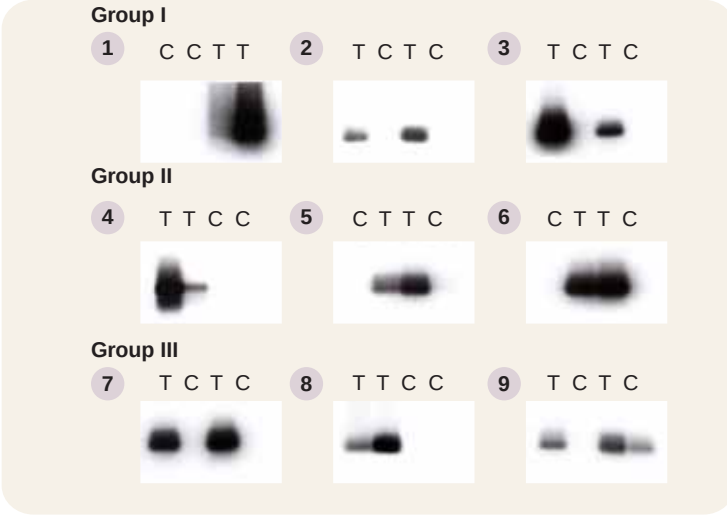
Conclusions

These studies confirm that it is possible to transfer anti-arthritic genes to human, rheumatoid joints and to express those genes intra-articularly. The procedure appears safe and the patients accepted it well. Preliminary data suggest marked clinical improvement in response to gene transfer, but this will need to be confirmed in larger, Phase II and III trials.

Future perspective

The *ex vivo* protocol reported here shows considerable biological promise, but there are concerns that it may not be a cost-effective way to treat large numbers of individuals. In particular, the harvest, culture, genetic modification, testing and reimplantation of autologous cells is very tedious, invasive and expensive. Replacing

Figure 4. Transgene expression in genetically modified joints.



RNA was extracted from the tissue retrieved by joint replacement surgery and subjected to a reverse-transcriptase polymerase chain reaction procedure that discriminated between interleukin-1 receptor antagonist transcripts from endogenous and transferred genes. The reaction products were separated by agarose gel electrophoresis, subjected to Southern blotting and visualized by autoradiography. Each of the four metacarpophalangeal joints from each subject (subjects 1–9) from the low (group I; subjects 1–3), medium (group II; subjects 4–6) and high (group III; subjects 7–9) dose groups are indicated as receiving T or C cells.
C: Control; T: Transduced.
Reproduced with permission from Evans CH, Robbins PD, Ghivizzani SC *et al.*: Gene transfer to human joints: progress toward a gene therapy of arthritis. *Proc. Natl Acad. Sci. USA* 102(24), 8698–8703 (2005).

autologous synovial fibroblasts with dermal fibroblasts offers one way to simplify tissue procurement, but unless a universal donor line of allograft transduced cells can be developed, the economic barriers will remain. There are suggestions that mesenchymal stem cells are amenable to allografting and it would be interesting to evaluate them in the present context [16].

In vivo gene delivery by the direct, intra-articular injection of suitable vectors provides a more expeditious route for arthritis gene therapy [17,18]. At the present state of the art, adeno-associated virus (AAV) seems the best suited vector for this purpose [3,19]. A Phase I clinical trial using AAV to deliver an etanercept cDNA to human joints with inflammatory arthritides has just been completed. The data have not been published yet, but according to the company website concerned, the procedure was well tolerated and 9 out of 11 participants showed a clinical response [101]. However, because it is difficult and expensive to make large amounts of clinical grade AAV, cost may also become a limiting factor for AAV-based gene therapies. Considerable effort is being devoted to overcoming this obstacle.

As indicated by the AAV etanercept trial, there are many transgenes other than IL-1Ra that hold promise for the gene therapy of RA [3]. We can expect several of these to be brought forward into clinical trials as the field progresses.

Executive summary

Preclinical data

- Gene therapy successfully treats animal models of rheumatoid arthritis (RA) and osteoarthritis (OA).

Human protocols

- The intra-articular delivery of a cDNA encoding the interleukin-1 receptor antagonist (IL-1Ra) has been evaluated in two clinical trials.
- In the first trial, an *ex vivo* protocol used a retrovirus vector to deliver IL-1Ra cDNA to metacarpophalangeal joints of nine subjects with RA. After 1 week, the joints were surgically removed during sialistic implant arthroplasty.
- In the second trial, a similar protocol was used in the joints of two subjects. After 1 month, the joints underwent surgical synovectomy.

Clinical findings

- Studies confirmed that genes could be safely transferred to human rheumatoid joints and expressed intra-articularly.
- The second study showed marked clinical improvement in response to IL-1Ra gene therapy.
- These promising data encourage further development of gene therapies for both RA and OA.

Conclusions

- Although *ex vivo* gene delivery appears effective, *in vivo* gene delivery will expedite the arrival of convenient, cost-effective gene therapies for arthritis.
- At present, recombinant adeno-associated virus vectors provide the best opportunities for achieving this.

Previously, we have argued that preclinical studies on RA gene therapy have convincingly established proof-of-concept [3] and the emphasis should now be on conducting clinical trials. These, however, are expensive and it is difficult for academic researchers to fund such studies through the usual channels. Under these conditions, it is customary to turn to industry for support; however, the success of enbrel, remicade, humira and other biological agents has greatly reduced the enthusiasm for investing in the development of gene therapy, even though the former are incompletely effective, require frequent invasive dosing and continue to raise concerns regarding side effects. There is a certain level of interest in arthritis gene therapy among small biotechnology companies, but most of these have insufficient resources for continuing beyond Phase I trials.

However, the economic logic changes when discussing OA, where there is a huge, unmet clinical and market need [4,20]. As OA lacks important extra-articular components and affects a limited number of joints, it may be particularly well suited to intra-articular gene therapy. Local gene therapy using IL-1Ra cDNA has shown efficacy in experimental models of OA in dogs, rabbits and horses, and could improve the treatment of this disease in human and veterinary medicine [4]. Thus, although arthritis gene therapy has its origins in the context of rheumatoid disease, its greatest application could eventually be in the treatment of OA. Given the large patient population and absence of competing therapies, OA could indeed be a major market for gene therapy in the next 5–10 years.

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101. Clinical trials from Targeted Genetics www.targetedgenetics.com/trials/rheumatoid-arthritis.php

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