Gene Therapy: What Have We Accomplished and Where Do We Go from Here?

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ABSTRACT. As a potential treatment for arthritis, gene transfer should be viewed within the context of biological therapy. Its particular strengths include the ability to deliver therapeutic gene products, both RNA and protein, to specific cells or tissues in a targeted, sustained, and potentially regulated, cost-effective fashion. An expanded definition of gene therapy includes the delivery of noncoding nucleotide sequences that act, for example, as decoy molecules. Considerable experimental progress has been made in the preclinical development of gene therapies for arthritis. Indeed, there is overwhelming proof of principle in animal models of rheumatoid arthritis (RA) and accumulating evidence of efficacy in animal models of osteoarthritis (OA). Early-phase human clinical trials have been successfully conducted and others are in progress. Additional research is necessary to optimize gene transfer technologies and achieve regulated transgene expression. However, the most urgent need is for interventional studies in human disease and the funding with which to implement them. (J Rheumatol 2005;32 Suppl 72:17-20)

Key Indexing Terms:
GENE THERAPY RHEUMATOID ARTHRITIS OSTEOARTHRITIS

Although the arthritides are not monogenic in origin, they may be treated by gene therapy. In this context, gene transfer becomes a biological method of delivering therapeutic gene products in an optimized fashion. The potential advantages of this are several, including the ability for site-specific, sustained, and, ultimately, regulated expression of antiarthritic molecules in a safe and cost-effective manner.

The preclinical development of arthritis gene therapy can be viewed as answering the following questions[1]:

- Where to put the therapeutic genes?
- How to get them there?
- Which genes to transfer?
- How to achieve appropriate regulation of gene expression?
- How to establish safety?

Clinical development requires the design and implementation of phase I, II, and III protocols. Within this schema, there has been considerable progress.

WHAT WE HAVE ACCOMPLISHED?

Where to put therapeutic genes?

In general terms, there are 3 potential targets: individual diseased joints (local therapy), sites where a secreted gene product has free access to the systemic circulation (systemic delivery), and cells with the ability to home to sites of disease activity (facilitated local delivery).

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Systemic delivery is probably the most achievable of these options as intramuscular gene transfer, for example, is already known to support longterm transgene expression in experimental animals[2]. Compared to present methods of delivering recombinant antiarthritic proteins, systemic gene delivery offers the advantages of requiring infrequent dosing at lower cost. However, side effects would not be reduced and, with present technology, it is easier to cease administration of a protein than switch off transgene expression. Moreover, systemic gene delivery fails to take full advantage of gene transfer's major strengths, namely, the opportunity for targeted delivery and localized gene expression. These are particularly valuable for the genetic treatment of OA.

The concept of gene transfer to cells with useful tropisms is very attractive. It has the dual advantages of using cells, particularly lymphocytes[3,4] and dendritic cells[5,6], that not only migrate selectively to sites of disease activity but also participate in restoring immune balance. Data from animal models are encouraging in terms of efficacy, but the safety of introducing genetically modified immunocompetent cells into the body requires scrutiny. Moreover, present approaches use *ex vivo* methods, and these are unlikely to be cost-effective. This approach is better suited to RA than OA.

Local gene delivery to individual diseased joints was the original concept for a gene therapy of arthritis[7,8], and it has stood the test of time, leading to several clinical trials. By achieving sustained therapeutic concentrations of transgene products selectively within the joint, this approach accomplishes something that is not reasonably possible by alternative technologies. It also promises to be safe and cost-effective. Its perceived disadvantages in RA of having to treat each joint individually and failing to address extraarticular manifestations of disease may

be offset by the type of interarticular communication noted in the "contralateral effect." This refers to the observation of Ghivizzani, *et al*[9] that gene transfer to one joint of an animal with bilateral disease led to amelioration of both the treated and contralateral, untreated joint. Further research suggested that dendritic cells might be responsible for the effect[10], leading to the use of genetically modified dendritic cells as an alternative gene therapy strategy[5]. Local gene delivery is the approach of choice in OA.

How to get them there?

Various viral and nonviral vectors have been evaluated as suitable gene transfer vehicles for arthritis gene therapy[11,12]. Moloney-based retroviruses have been successfully used for *ex vivo* delivery strategies and, until recently, were perceived to be safe. The first cases of insertional mutagenesis have altered this perception and the future is unclear. Research is being directed towards developing retroviruses with predictable integration sites, but these are not yet available. Meanwhile, they remain very useful in preclinical studies as they are straightforward to produce and express no viral proteins.

A drawback of Moloney-type viruses is their inability to transduce nondividing cells, a circumstance that limits their use to *ex vivo* strategies. This limitation has been removed by development of a different type of retrovirus vector based upon lentiviruses. Direct injection of recombinant lentivirus into the joint is a very efficient way to transfer genes to synovium[13,14]. There are no obvious adverse sequelae, and longterm transgene expression can be achieved. Nevertheless, there still remains the issue of insertional mutagenesis. Moreover, many types of lentiviral vectors are derived from human immunodeficiency virus (HIV). Although recombinant HIV-based vectors cannot cause acquired immunodeficiency syndrome, they come with considerable psychological baggage. There are alternative lentivirus vectors based upon, for example, feline immunodeficiency virus or equine infectious anemia virus, but the uncertain properties of these viruses when introduced into human cells raise additional concerns.

Unlike their wild-type counterparts, recombinant adeno-associated virus (AAV) vectors do not integrate but persist in the nucleus as large concatamers. There is disagreement whether AAV provide longterm transgene expression in joints[15-17]. However, it is presently considered to be a safe vector for human gene therapy, and it has recently entered the clinic in a phase I trial for RA. Production of large amounts of AAV remains problematic and its poor infectivity often requires the use of very high multiplicities of infection. Different serotypes of AAV are presently being evaluated for their abilities to infect various types of cells, and this may lead to greater efficiencies.

Recombinant adenovirus has found widespread success as an experimentally useful vector for arthritis gene therapy studies[9]. First and second generation vectors are unlikely to find clinical application, because of their immunogenic properties. Gutted adenovirus vectors offer better prospects in this regard, but transgene expression using such viruses is low.

Nonviral vectors have generally proved disappointing in arthritis gene therapy strategies requiring sustained transgene expression at reasonably high levels[11,18], although recent data suggest that electroporation holds promise[19]. Nonviral gene transfer may, however, provide a sufficient level and duration of transgene expression for approaches requiring apoptosis[20]. Moreover, decoys and antisense molecules show efficacy without viral delivery.

Which genes to transfer?

Numerous transgenes work in animal models of RA[21]. These can be loosely grouped as cytokine antagonists, immunomodulators, apoptotic agents, inhibitors of angiogenesis, and intracellular mediators. In addition, transfer of interleukin 1Ra (IL-1Ra) cDNA to the synovial linings of joints is chondroprotective in canine, lapine, and equine models of OA[22].

How to achieve appropriate regulation of transgene expression?

Arthritic diseases are chronic, and RA in particular is subject to flare and remission. Thus a successful gene therapy is likely to require longterm, regulated transgene expression or facile readministration. The issue of longterm gene expression has been best studied in the synovium, where recent data suggest that this can be achieved when an autologous transgene is delivered by an immunologically silent vector[13].

In the absence of longterm transgene expression, there has been little incentive to study regulated transgene expression in the context of arthritis gene therapy. A variety of inducible systems exist. Many, such as the commonly used systems based upon the Tet repressor, suffer from the involvement of nonhuman proteins. These will permit short term experimental studies in laboratory animals, but may prove antigenic in humans. An exception is the rapamycin-based system, which shows considerable promise[23].

As an alternative, there are possibilities for endogenous regulation of transgene expression using inducible promoters. Impressive results have been reported with the construct of Varley, *et al*[24,25]. This uses inducible promoters that regulate expression of acute phase proteins, with amplification by the TAT transactivator of HIV.

Is gene therapy safe?

Because arthritic diseases are not immediately life-threatening, their treatment by gene therapy raises considerable

Table 1. Clinical trials in the gene therapy of arthritis.

Indication and Phase	Gene	In/Ex Vivo	Vector	PI or Company	Status
RA phase I	IL-1Ra	Ex	Retrovirus	Evans and Robbins	Closed
RA phase I	IL-1Ra	Ex	Retrovirus	Wehling	Closed
RA phase I	HSV-tk	In	Plasmid	Roessler	Closed
RA phase I	NF-κB decoy	In	Oligo	Tomita	Open
RA phase I	TNF antisense	In	Oligo	Isis	Open
RA phase I	TNFsR:IgG	In	AAV	Targeted Genetics	Open¸
RA phase II	IL-1Ra	Ex	Retrovirus	Evans	Pending
OA phase I	IL-1Ra	In	AAV	Evans	Pending

PI: principal investigator; IL: interleukin; HSV: herpes simplex virus; NF-κB: nuclear factor κB; TNF: tumor necrosis factor; TNFsR; TNF soluble receptor.

safety concerns. Of these, issues related to the use of viral vectors predominate, especially after the well publicized death of Jesse Gelsinger in 1999 and the more recent occurrence of leukemia in children receiving gene therapy for X-linked severe combined immunodeficiency disease.

No arthritis gene therapy clinical trial has reported adverse events, but such trials are few in number and of short duration. AAV is emerging as a vector of choice for future clinical studies, because of its perceived safety. There are renewed safety issues surrounding the use of the more powerful retroviral vectors, and, in the continued absence of targeted insertion, such vectors may need to be engineered to contain inducible suicide genes.

One of the advantages of local gene delivery to joints is that adverse events can be contained and managed. Targeting the synovium provides the opportunity for synovectomy, should it be necessary to remove the genetically modified cells.

CLINICAL TRIALS

There have been 3 phase I studies for RA and 3 more are in progress; a phase II protocol for RA and a phase I for OA await funding (Table 1). Two of the 3 completed studies involved the retroviral, *ex vivo* transfer of the IL-1Ra cDNA to rheumatoid metacarpophalangeal joints one week or one month before total joint replacement or synovectomy[26,27]. A total of 15 patients were treated in these 2 studies. They confirmed that it is possible to transfer genes to human rheumatoid joints in a manner that is safe and acceptable to patients. A phase II study is pending. The third phase I protocol transferred the thymidine kinase gene of herpes simplex virus to the synovium by the intraarticular injection of plasmid. This rendered the transfected cells sensitive to subsequent administration of ganciclovir, the aim being to achieve a genetic synovectomy[20]. This trial was closed after one subject was treated safely in this manner, because of a failure to recruit.

Where do we go from here?

There is overwhelming proof of principle that gene therapy works in animal models of RA. In the author's view, there is little point in devoting resources to embellishing this fact. Rather, the most pressing need is for further human studies. Such studies are expensive and complex, but a necessary investment if we are to develop gene-based treatments that work in human disease as opposed to showing promise in the experimental diseases of laboratory animals.

Because of the success of recombinant proteins such as infliximab and etanercept, some of the urgency has gone out of developing new treatments for RA. However, OA remains very common and, in many cases, resistant to pharmacological control. It costs the US economy roughly $100 billion per annum, a figure that will rise as the population ages and becomes fatter. Promising preclinical data suggest a future role for gene therapy in addressing OA[22], and the first clinical protocol is waiting in the wings.

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