

Review

Lessons learned from gene transfer approaches

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Abstract

Recent technological advances allow the transfer of genes to the synovial lining of joints. As well as opening novel opportunities for therapy, these techniques provide valuable new tools for the study of synovitis and other aspects of the biology of joints in health and disease. This article reviews briefly the results of experiments in which selected genes have been transferred to the knee joints of healthy rabbits and rabbits with antigen-induced arthritis.

Keywords: animal model, cartilage, cytokine, gene therapy, growth factor, rheumatoid arthritis, synovium

Introduction

One approach to the treatment of intra-articular pathologies involves the transfer of genes to synovium and their intrasynovial expression [1]. The development of technologies with which to do this not only opens new therapeutic possibilities but also permits the study of selected gene products within the synovial microenvironment. Here we review briefly the results obtained from such investigations.

Most of these data have been obtained using the rabbit knee as a model. This joint has the advantage of being large enough for efficient intra-articular injection and serial lavage. Moreover, after euthanasia the various intra-articular tissues can be dissected and independently studied *ex vivo*.

We utilize gene delivery to the joint in two complimentary approaches to the study of joint diseases. In the first, genes whose products have been associated with arthritis are introduced into the synovium of a normal joint to determine whether their gene products can provoke synovitis or other joint disturbances. In the second, the genes

are introduced into inflamed joints to determine whether their products have anti-inflammatory properties. Antigen-induced arthritis (aia), using ovalbumin as the inciting antigen, is often employed for these experiments.

Gene transfer to synovium

Previous research has demonstrated the experimental utility of two methods for delivering genes to the synovial lining of joints, *ex vivo* transfer using a retrovirus and *in vivo* delivery using an adenovirus (Table 1) [2–4]. The former approach has been used in a human clinical trial [5], but is cumbersome and tedious, and selectively targets type B synoviocytes. For these reasons, most of the experimental studies described here have involved *in vivo* gene delivery by direct, intra-articular injection of adenoviral vectors. This method is not only simpler but also transduces types A and B synoviocytes as well as various leukocyte populations within the joint. These studies have to be performed carefully, because the adenoviruses themselves may provoke an inflammatory response. However, it is possible to titrate downwards the amount of adenovirus injected into the joint

aia = antigen-induced arthritis; BMP = bone morphogenetic protein; FasL = Fas ligand; GAG = glycosaminoglycans; hIL = human interleukin; IGF = insulin-like growth factor; IL-1Ra = interleukin-1 receptor antagonist; NO = nitric oxide; NOS = nitric oxide synthase; TGF = transforming growth factor; TNF = tumor necrosis factor; TNFRa = tumor necrosis factor receptor antagonist; vIL-10, viral interleukin 10; WBC = white blood cell.

Table 1**Ex vivo and in vivo gene transfer to the synovial lining of rabbits' knee joints***

| | <i>In vivo</i> | <i>Ex vivo</i> |
|------------------------|--|---------------------|
| Level of expression | High | Low-medium |
| Duration of expression | ~ 2 weeks | Up to 6 weeks |
| Transduced cells | Types A+B synoviocytes Various leukocytes | Type B synoviocytes |
| Laboriousness | Low | High |
| Inflammatory response | Present | Absent |

*This comparison is based upon *ex vivo* gene transfer using Moloney-based retrovirus, and *in vivo* gene transfer using first generation adenovirus.

such that an experimentally useful level of gene expression is achieved with only minimal inflammation resulting from the virus itself. The intra-articular expression of transgenes following adenoviral delivery tends to be high initially, but then declines to undetectable levels within about 2 weeks. Because of this, and the nature of aia in the rabbit knee, most experiments have studied the acute inflammatory reaction occurring during the first week of disease. This period of time also avoids complications that would arise from a primary immune response to the vector or the transgene products, most of which are heterologous proteins.

Gene transfer to the normal synovium

Ex vivo transfer of the interleukin-1 β gene

The first experiments of this kind were conducted by *ex vivo* transfer of a human interleukin (hIL)-1 β complementary (c)DNA [6]. Because the native molecule is not secreted efficiently, a leader sequence taken from the parathyroid hormone was fused to the N-terminus of the molecule. This molecule proved extraordinarily potent. Indeed in the first experiment where 10⁷ autologous cells expressing IL-1 β were returned to one knee joint, all the rabbits died or had to be euthanized.

In subsequent experiments, fewer cells were returned to the joint. Following intra-articular injection of 10⁶ transduced cells, approximately 200 pg hIL-1 β could be lavaged from the joints for up to 2 weeks, after which gene expression abruptly stopped. During this 2-week period, the rabbits developed fevers, lost weight, and their erythrocyte sedimentation rates rose dramatically. Knee joints receiving the hIL-1 β construct were massively swollen and contained a voluminous, caseinous synovial fluid. Synovia were enormously hypertrophic and hyperplastic, not only invading the adjacent cartilage and bone but also penetrating the capsule into the adjacent muscle, areas of which became necrotic. It is also remarkable how all non-erosive components of these pathologies returned to normal once transgene expression was lost.

Later studies revealed that the transfer of as few as 10⁵ IL-1 β ⁺ cells caused considerable intra-articular pathology despite the absence of detectable hIL-1 β in the lavage fluid. This observation cautions against drawing strong conclusions concerning the pathophysiological roles of potential mediators based upon their synovial fluid concentrations.

Previous attempts to define intra-articular responses to IL-1 β had relied upon intra-articular injection or pumps to deliver recombinant protein [7,8]. In neither case were the sequelae remotely as dramatic as those observed following transfer of the IL-1 β gene. The greater potency of the gene transfer approach may not only result from its ability to deliver IL-1 β in a sustained fashion, but also from the production of an authentically processed protein by synoviocytes within the synovial microenvironment.

In vivo gene transfer

A variety of genes have been evaluated by *in vivo* adenoviral delivery (Table 2). Expression of a murine tumor necrosis factor (TNF) cDNA within the synovium of the rabbit knee produced moderate inflammatory infiltrates, which were particularly rich in polymorphonuclear leukocytes (our unpublished data, 1998). The release of glycosaminoglycans (GAG) from the articular cartilage was modestly elevated, while GAG synthesis was strongly depressed. When expressed in combination with an IL-1 β transgene, TNF caused high levels of apoptosis in the synovium. This suggests that such a combination may limit synovitis by inducing cell death.

Growth factor genes are being evaluated for their ability to protect articular cartilage from degradation, as well as to enhance the repair of damaged cartilage. As discussed in [9], these genes may be delivered to synovium or to the cartilage itself. Transfer of a human transforming growth factor (TGF)- β ₁ gene to synovium provoked a massive hypertrophy and fibrosis of not only the injected joint, but also the soft tissues of the entire leg (our unpublished data, 1998). This proved fatal in several animals. When less virus was administered, pathology was restricted to the injected knee and included thickening and fibrosis of the synovium. This suggests that TGF- β may be a mediator of arthrofibrosis. Interestingly, the inflammatory infiltrate that often follows the intra-articular injection of adenoviral particles was virtually absent when the TGF- β ₁ gene was present.

Of particular interest was the formation of intrasynovial cartilage. Recent data suggest that cells within the synovium have the potential to differentiate into chondrocytes and to form cartilage. Our findings indicate that TGF- β can trigger this pathway and, in this way, lead to the human condition synovial osteochondromatosis [10].

Synovial delivery of genes encoding insulin-like growth factor (IGF)-1 and bone morphogenetic protein (BMP)-2,

Table 2

Intra-articular effects of various genes following direct delivery to the synovial lining of normal rabbits knees

| Gene | Effect* | | | |
|---------------------------------|---------|-----------|---------------------|-----------|
| | Edema | Synovitis | Cartilage synthesis | Breakdown |
| TNF | + | ++ | -- | ++ |
| TGF-β ₁ [†] | +++ | + | ± | ++ |
| IGF-1 | 0 | 0 | ++ | 0 |
| BMP-2 | 0 | 0 | ++ | 0 |
| iNOS [‡] | 0 | + | 0 | 0 |

*Effects are graded on a scale from 0 = no effect to +++ = large stimulatory effect to --- = large inhibitory effect. [†]Delivery of the TGF-β₁ gene was associated with the intrasynovial formation of cartilage. [‡]Delivery of the iNOS gene was associated with neovascularization of synovium. TNF, tumor necrosis factor; TGF, transforming growth factor; IGF, insulin-like growth factor; BMP, bone morphogenetic protein; iNOS, inducible nitric oxide synthase.

in contrast to the TGFβ gene, produced a modest elevation in cartilage GAG synthesis in the absence of major synovial changes (our unpublished data, 1998).

Nitric oxide (NO) is a radical with a physiological half-life of several seconds. Although there is literature to suggest an important role for this molecule in inflammation and joint disease [11], experimental studies have been hampered by various practical limitations. Among them is the near impossibility of administering NO to experimental animals in a pharmacologically useful manner. Transfer of genes encoding NO synthase solves this problem, and serves as a particularly good example of the value of the gene delivery approach to studying disease.

Intrasynovial expression of a cDNA encoding human, inducible NO synthase (iNOS or NOS-2), produced remarkably few intra-articular changes beyond a mild influx of leukocytes into the synovium and the joint space. This infiltrate was unusual in containing predominantly monocytes and macrophages. The relative absence of neutrophils may reflect the ability of NO to inhibit the expression of neutrophil-specific adhesion molecules on the endothelial lining of the synovial vasculature [12]. The synovium was slightly thickened, and there was evidence of considerable neo-vascularization. No effects were seen on the turnover of cartilage proteoglycans (our unpublished data, 1998).

Gene transfer to the inflamed synovium

Ex vivo transfer of the interleukin-1 receptor antagonist (IL-1Ra)

Delivery of a cDNA encoding human IL-1Ra one day after induction of aia had no effect on synovitis, although it reduced the influx of leukocytes into the joint space and

Table 3

Intra-articular effects of various genes following direct delivery to the synovial lining of rabbit knees with antigen-induced arthritis

| Gene | Effect* | | | | |
|----------------|---------|-----------|-----|-----------|------------------|
| | Edema | Synovitis | WBC | Cartilage | |
| | | | | Synthesis | Deg ⁿ |
| IL-1Ra | 0 | 0/- | - | NT | - |
| TNFRa | 0 | 0 | 0/- | NT | 0 |
| IL-1Ra + TNFRa | - | - | - | NT | - |
| vIL-10 | - | - | - | + | - |
| IGF-1 | 0 | 0 | 0 | + | 0 |

*0, No effect; -, inhibition; +, stimulation; NT, not tested; WBC, white blood cell infiltrate into the synovial fluid; Degⁿ, degradation; IL-1Ra, interleukin-1 receptor antagonist; TNFRa, tumor necrosis factor receptor antagonist; vIL-10, viral interleukin-10; IGF, insulin-like growth factor.

strongly protected the articular cartilage. For unknown reasons transgene expression was higher in joints with aia than in normal joints [13]. Although synovitis was unaffected in this rabbit model, *ex vivo* transfer of human IL-1Ra genes did suppress synovitis in streptococcal cell wall arthritis in rats [14] and collagen-induced arthritis in mice [15]. *Ex vivo* delivery of the IL-1Ra gene to joints is presently the subject of a human clinical trial [5].

In vivo gene transfer

Adenoviral vectors have been used to transfer a number of additional potentially anti-inflammatory genes to the synovia of rabbits with aia (Table 3). A gene encoding bivalent IL-1 soluble type I receptor inhibited the influx of leukocytes into the joint space and protected the articular cartilage. Synovitis was moderately reduced [16].

Delivery of a gene encoding bivalent TNF soluble type I receptor had very little effect beyond a mild reduction in the inflammatory infiltrate into the joint space. Remarkably, a combination of both IL-1 and TNF soluble receptor genes not only protected cartilage and reduced the leukocytic influx into the joint space, but also inhibited joint swelling and strongly reduced the synovitis [16].

The viral (v)IL-10 gene was even more potent than the combination of IL-1 and TNF soluble receptors. Alone, it strongly reduced swelling, synovitis and leukocytic infiltration while protecting the articular cartilage [17]. A similar effect has been noted in the paws of mice with collagen-induced arthritis [18].

In vivo delivery of human Fas ligand (FasL) was associated with large regions of cellular apoptosis within the

synovium. It is not yet clear whether the level of cell killing observed under these circumstances is sufficient to produce a significant anti-arthritic effect in this model (our unpublished data, 1998). Adenoviral delivery of the FasL gene does, however, ameliorate disease in murine collagen-induced arthritis [19].

The IGF-1 gene had no effect on synovitis or other inflammatory components of aia, but was able to increase the synthesis of GAG by cartilage without influencing breakdown. Expression of an iNOS gene within the joints of rabbits with aia had no effect on synovitis or other intra-articular pathology (our unpublished data, 1998).

The contralateral effect

While performing these experiments, Ghivizzani *et al* [16] made an unexpected observation: injection of adenovirus carrying the soluble IL-1 receptor into one knee joint of animals with bilateral aia ameliorated disease not only in the injected knee but also in the contralateral knees which had received only saline or a marker gene. When a soluble TNF receptor gene was co-administered, this 'contralateral effect' was even stronger. The contralateral effect was particularly powerful when using the vIL-10 gene [17]. A similar effect occurs in murine collagen-induced arthritis [18], and several other groups have reported similar findings suggesting that this is not some peculiarity of rabbit a.i.a [20].

The mechanism of the contralateral effect may reside with the ability of leukocytes to traffic between joints. Following injection of adenoviruses carrying marker genes into one knee joint of animals with bilateral disease, it is possible to recover cells expressing those marker genes from the contralateral knee joints and draining lymph nodes but not from other organs or the peripheral blood [16,17]. The identification of these cells and their possible role as mediators of the contralateral effect are under investigation.

Conclusions

The ability to transfer genes to the synovium and other tissues of interest offers unique opportunities for studying the biology of joints in health and disease. The data obtained by these means will aid the development of novel therapeutic approaches, irrespective of their direct application to gene therapy.

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