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.
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-1β gene

The first experiments of this kind were conducted by ex vivo transfer of a human interleukin (hIL)-1β 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-1β 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-1β 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-1β 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 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-1β⁺ cells caused considerable intra-articular pathology despite the absence of detectable hIL-1β 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-1β 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-1β gene. The greater potency of the gene transfer approach may not only result from its ability to deliver IL-1β 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-1β 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)-β₁ 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-β₁ 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,
in contrast to the TGF-β 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 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].

**Table 2**

| Gene       | Edema | Synovitis | Cartilage synthesis | Breakdown |
|------------|-------|-----------|---------------------|-----------|
| TNF        | +     | ++        | -                   | ++        |
| TGF-β1     | +++   | +         | ±                   | ++        |
| 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-β 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.

**Table 3**

| Gene            | Edema | Synovitis | WBC             | Cartilage |
|-----------------|-------|-----------|-----------------|-----------|
| IL-1Ra          | 0     | 0/–       | –               | NT        |
| TNFRa           | 0     | 0         | 0/–             | NT        |
| 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; Deg, degradation; IL-1Ra, interleukin-1 receptor antagonist; TNFRa, tumor necrosis factor receptor antagonist; vIL-10, viral interleukin-10; IGF, insulin-like growth factor.
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 aia [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.

References

1. Evans CH, Ghivizzani SC, Kang R, et al. Gene therapy for rheumatic diseases. Arthritis Rheum 1999, 42:1–15.
2. 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 1992, 11:227–231.
3. Roesler BJ, Allen ED, Wilson JM, Hartman JW, Davidson BL. Adenoviral-mediated gene transfer to rabbit synovium in vivo. J Clin Invest 1993, 92:1095–1092.
4. Nita I, Ghivizzani SC, Galea-Lauri J, et al. Direct gene delivery to synovium: an evaluation of potential vectors in vitro and in vivo. Arthritis Rheum 1996, 39:820–828.
5. Evans CH, Robbins PD, Ghivizzani SC, Hendron JH, Kang R: Clinical trial to assess the safety, feasibility and efficacy of transferring a potentially anti-arthritic cytokine gene to human joints with rheumatoid arthritis. Human Gene Ther 1996, 7:1261–1280.
6. Ghivizzani SC, Kang R, Georgescu HI, et al. Constitutive intra-articular expression of human IL-1β following gene transfer to rabbit synovium produces all major pathologies of human rheumatoid arthritis. J Immunol 1997, 159:3604–3612.
7. Pettipher ER, Higgs GA, Henderson B. Interleukin-1 induces leukocyte infiltration and cartilage proteoglycan degradation in the synovial fluid. Proc Natl Acad Sci U S A 1986, 83:8749–8753.
8. Feige U, Karbowici C, Rordorf-Adam C, Pataki A: Arthritis induced by continuous infusion of hIL-1ra into the rabbit knee joint. Int J Tissue React 1989, 11:225–238.
9. Evans CH, Robbins PD: Potential treatment of osteoarthritis by gene therapy. In: Rheumatic Disease Clinics of North America. Edited by Brandt K. Philadelphia: WB Saunders Co, 1999, 25:333–344.
10. Milgram JW. Synovial osteochondromatosis: a histopathological study of thirty cases. J Bone Joint Surg 1977, 59A:792–801.
11. Stefanovic-Racic M, Sladder J, Evans CH: Nitric oxide and arthritis. Arthritis Rheum 1993, 36:1036–1044.
12. Niu XF, Ibbotson G, Kubes P. A balance between nitric oxide and oxidants regulates mast cell-dependent neutrophil-endothelial cell interactions. Circ Res 1996, 79:992–999.
13. Otani K, Nita I, Maaculay W, Georgescu HI, Robbins PD, Evans CH: Suppression of antigen-induced arthritis by gene therapy. J Immunol 1996, 156:3558–3562.
14. Makarov SS, Iaen JC, Johnson WA, et al. Suppression of experimental arthritis by gene transfer of interleukin-1 receptor antagonist. J Immunol 1999, 163:402–406.
15. Bakker AC, Joosten LAB, Amtz OJ, et al. Prevention of murine collagen-induced arthritis in the knee and ipsilateral paw by local expression of human interleukin-1 receptor antagonist. Proc Natl Acad Sci U S A 1996, 93:402–406.
16. Ghivizzani SC, Lechman ER, Kang R, et al. Direct adenoviral-mediated transfer of IL-1 and TNF-α soluble receptors to rabbit knees with experimental arthritis has local and distal anti-arthritic effects. Proc Natl Acad Sci U S A 1998, 95:4613–4618.
17. Lechman ER, Jaffurs D, Ghivizzani SC, et al. Direct adenoviral gene transfer of vIL-10 to rabbit knees with experimental arthritis ameliorates disease in both injected and contralateral knees. J Immunol 1999, in press.
18. Whalen JD, Lechman ER, Carlos CA, et al. Adenoviral transfer of the viral IL-10 gene peripherically to mouse paws suppresses development of collagen-induced arthritis in both injected and un.injected paws. J Immunol 1999, 162:3625–3632.
19. Zhang H, Yang Y, Horton JL, et al. Amelioration of collagen-induced arthritis by CD95 (Apo-1/Fas)-ligand gene transfer. J Clin Invest 1997, 100:195–1967.
20. Evans CH, Rediske JJ, Abramson SB, Robbins PD: Joint efforts: tackling arthritis using gene therapy. Mol Med Today 1999, 5:148–151.

Author addresses: Christopher H Evans (Orthopaedic Surgery and Molecular Genetics & Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA); Steven C Ghivizzani, Eric R Lechman, Zhebao Mi, Daniel Jaffurs and Paul D Robbins (Molecular Genetics & Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA)

Correspondence: C H Evans, PhD, DSc, University of Pittsburgh School of Medicine, Department of Orthopaedic Surgery, 200 Lothrop Street, Room C313 PUH, Pittsburgh, PA 15213, USA. Tel: +1 412 648 1092; fax: +1 412 648 8412; e-mail: cevans@vms.cis.pitt.edu