

Meeting report

Gene therapy moves forward – The Second International Meeting on Gene and Cell Therapies of Arthritis and Related Disorders, 17–18 May 2001, Montpellier, France

Paul D Robbins*, Christian Jorgensen† and Christopher H Evans‡

* Department of Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, PA, USA

† Lapeyronie University Hospital, 34295 Montpellier, France

‡ Center for Molecular Orthopaedics, Harvard Medical School, Boston, MA, USA

Correspondence: Paul D Robbins, PhD, Department of Molecular Genetics and Biochemistry, W1246 BST, University of Pittsburgh, Pittsburgh, PA 15261, USA. Tel +1 412 648 9268; fax +1 412 383 8837; e-mail probb@pitt.edu

Received: 16 July 2001

Accepted: 16 July 2001

Published: 31 July 2001

Arthritis Res 2001, **3**:289–292

© 2001 BioMed Central Ltd

(Print ISSN 1465-9905; Online ISSN 1465-9913)

Abstract

The field of gene therapy for bone and joint disorders has grown considerably over the last two and a half years. Investigators have shown that *ex vivo* or *in vivo* gene transfer is highly effective in blocking arthritis or facilitating repair of damaged cartilage or bone. The feasibility of applying gene therapy for the treatment of arthritis in humans has also been demonstrated. Thus, gene therapy appears poised to make significant contributions to the clinical treatment of joint and bone diseases in the near future.

Keywords: arthritis, bone, cartilage, clinical trial, gene therapy, vector

Introduction

The Second International Meeting on Gene and Cell Therapies of Arthritis and Related Disorders was held in Montpellier, France on May 17 and 18, hosted by Christian Jorgensen (Montpellier, France). The previous meeting was held in Washington, DC in December of 1998. What was clearly evident from the second meeting is that the field of gene therapy for bone and joint disorders has grown considerably over the last two and a half years in terms of both the number of investigators and the types of approaches being developed. Several investigators have shown that *ex vivo* or *in vivo* gene transfer is highly effective in blocking the development of arthritis or facilitating repair of damage cartilage or bone. The feasibility of applying gene therapy for the treatment of arthritis in humans was also demonstrated. Thus, gene therapy appears

poised to make significant contributions to the clinical treatment of joint and bone diseases in the near future.

Vectors

The rate-limiting step for any gene therapy application is the efficiency of gene transfer. Two approaches were discussed: direct, *in vivo* gene delivery and indirect, *ex vivo* gene transfer using synovial fibroblasts, chondrocytes, antigen-presenting cells (APCs), lymphocytes or mesenchymal stem cells (MSCs). Although a number of presentations discussed improvements in viral and nonviral vectors for direct gene transfer to joints or to bone defects, no vector yet appears ready to be used in *in vivo* clinical trials.

W van den Berg (Nijmegen, The Netherlands), P Robbins (University of Pittsburgh, USA) and T Oligino (University of

AAV = adeno-associated virus; Ad5 = adenovirus serotype 5; APC = antigen-presenting cell; BMP = bone morphogenetic protein; IGF = insulin-like growth factor; IL-1Ra = IL-1 receptor antagonist; MSC = mesenchymal stem cell; OPG = osteoprotegerin; RA = rheumatoid arthritis; SCID = severe combined immunodeficiency; sTNF-R = soluble tumor necrosis factor receptor; TGF = transforming growth factor.

Pittsburgh, USA) all presented evidence that adenoviral vectors are able to infect murine and rabbit synovial tissue as well as infecting infiltrating monocytes following injection into the joint space. Expression was transient, however, and the induction of neutralizing antibodies prevents repeat dosing. T Huizinga (Leiden University, The Netherlands) also demonstrated that synovial fluid from many rheumatoid arthritis (RA) patients contains neutralizing antibodies to adenovirus serotype 5 (Ad5), but that there are only low levels of neutralizing antibodies to certain serotypes of adenoviruses in the synovial fluids of RA patients. In particular, Ad35 is able to infect human synovial cells more efficiently than Ad5, but was not neutralized by pre-existing antibodies in synovial fluids. P Yeh (Aventis-Gencell, France) discussed strategies for targeting adenoviruses to particular cell types.

The properties of adeno-associated virus (AAV) were described by AM Douar (Gentho, France). AAV also appears to be able to infect cells in the joint space efficiently, resulting in prolonged gene expression (R Hirsch, University of Cincinnati, USA). Hirsch suggested, however, that AAV fails to transduce murine synovium but gives the appearance of doing so by transducing the adjacent muscle very effectively. Nevertheless, his data suggested that human synovial fibroblasts support transduction by AAV much more effectively than their murine counterparts. Given the initial success of clinical trials using AAV for gene transfer to muscle, there was optimism that AAV could be useful for intra-articular gene delivery as well as for systemic delivery of soluble proteins following intra-muscular injection. Both Oligino and Robbins reported that transgene expression following the intra-articular injection of an AAV vector was progressively lost during the course of 2–3 weeks. Expression could not be restored by readministration of the same vector, possibly because of the induction of neutralizing antibodies. It is not yet known whether such antibodies would be directed against the transgene or the virus. Immunotherapy for arthritis may be achieved through intramuscular injection of recombinant AAV that contains genes encoding anti-inflammatory cytokines. F Apparailly (Montpellier, France) reported the efficacy of viral IL-10 expression under the control of a tetOn inducible promoter using an AAV construct in collagen-induced arthritis, and M-C Boissier (Bobigny, France) also reported efficacy in this model using AAV-IL-4.

P Corbeau (Montpellier, France) discussed the development of lentiviral vectors and their ability to transduce cultures of human synovial fibroblasts, a finding in agreement with data showing that an HIV-based lentiviral vector was able to infect rat synovium following intra-articular IL-1 receptor antagonist (IL-1Ra) injection. Surprisingly high levels of intra-articular transgene expression were obtained (E Gouze, Harvard Medical School, USA).

Two laboratories (C Pitzalis, London, UK and P Robbins) have initiated studies to identify peptides that can target cells within the joint following systemic delivery or those that are able to transduce synovial cells following intra-articular delivery. These peptides could be used to deliver therapeutic proteins, drugs and possible plasmids or viral vectors to cells within the joint. Finally, the use of nonviral vectors was also presented. Although a number of nonviral vectors were able to transfect synovium *in vivo* following intra-articular injection, the duration of gene expression (of over 100 different nonviral formulations tested) was less than 1 week, with some of the formulations inducing inflammation (P Robbins). The ability to express soluble proteins in the long term from muscle by electroporation of the plasmid DNA encoding them was demonstrated by D Scherman (Aventis Gencell, France). The muscle thus represents an attractive target for regulated systemic expression of IL-1Ra, soluble tumor necrosis factor receptors (TNF-R) or IL-10, especially if naked plasmid DNA can be used as the vector. The same electroporation technology was used with remarkable success for *in vivo* gene delivery to chondrocytes, with up to 40% of chondrocytes from the patella expressing the transgene for 3 months (P Gillet, Vandoeuvre, France).

Arthritis therapy

The ability to treat animal models of arthritis effectively by gene delivery was confirmed by a number of investigators. Intra-articular gene transfer of IL-1 and TNF inhibitors such as sIL-R, IL-1Ra and sTNF-R, and immunosuppressive cytokines such as IL-4 and viral IL-10, as well as antagonists of NF- κ B (S Makarov, University of North Carolina, USA) were able to block inflammation as well as additional pathologies in murine, rat and rabbit models of arthritis. Importantly, the levels of these proteins necessary to confer a therapeutic effect following intra-articular gene delivery were far less than those needed for therapeutic efficacy following injection of recombinant protein. Interestingly, the van den Berg laboratory demonstrated that use of an IL-1 responsive promoter to drive IL-1Ra expression resulted in a better therapeutic effect *in vivo* compared to a constitutive promoter that gave higher expression. These results suggest that localized, regulated expression of IL-1Ra is highly effective in blocking disease. Taken together, the significant therapeutic effects observed following intra-articular gene transfer at relatively low doses of expression warrant the further development of this approach to the gene therapy of arthritis and related joint disorders. Adenoviral vectors may also be delivered systemically, and interesting results for the delivery of several immunomodulatory genes were described by E Quattrocchi (Kennedy Institute, UK).

As an alternative to treating arthritis by local, intra-articular gene delivery, several investigators presented the use of genetically modified immune system cells to modulate

disease progression. J Mountz (University of Alabama, USA) demonstrated that systemic delivery of antigen-presenting cells expressing Fas ligand and pulsed with type II collagen was able to suppress development of collagen-induced arthritis. In addition, systemic delivery of dendritic cells modified to express IL-4 was able to completely reverse established murine collagen-induced arthritis (P Robbins). The Fathman laboratory (A Nakajima, I Turner, Stanford University, USA) presented evidence that genetically modified, collagen-specific T cells could home to joints and confer a therapeutic effect in a murine arthritis model. Similarly, Y Chernajovsky (University of London, UK) demonstrated the ability to modify T cells to express a single chain anti-collagen antibody to target the cells to joints.

In addition to treating inflammation and joint swelling by gene transfer, several groups have focused on blocking or eliminating the hyperplastic synovium. U Müller-Ladner (University of Regensburg, Germany) presented results from a SCID (severe combined immunodeficiency) human model, showing that gene transfer of IL-1Ra and IL-10 to RA synovial fibroblasts blocks their invasion into normal cartilage as well as blocking cartilage degradation. Interestingly, the inclusion of sTNF-R gene transfer with IL-1Ra and IL-10 did not improve the therapeutic efficacy, but instead reduced it. S Gay (University Hospital Zurich, Switzerland) and T Pap (University Hospital Magdeburg, Germany) provided evidence that inhibition of cathepsins B and L and matrix metalloproteinase-1 expression using gene transfer of antisense or ribozymes to RA synovial cells was also therapeutic. Chondroprotection can also be achieved by overexpressing inhibitors of the proteinases that degrade the matrix of cartilage (W Van der Laan, Leiden University, The Netherlands; G Schett, Vienna, Austria), and by the transfer to chondrocytes of antiapoptotic genes (C Coumil, Vandoeuvre, France).

Several groups also examined the ability to eliminate hyperplastic synovium by intra-articular gene transfer of certain apoptotic proteins. H Zhang (University of Pennsylvania, USA) reported efficacy in human synovium implanted into SCID mice. T Oligino provided evidence that adenovirus-mediated gene transfer of several apoptotic genes, such as those encoding Fas ligand, TRAIL, p53 and granzyme B, resulted in extensive apoptosis in a rabbit model of arthritis. Interestingly, p53 was the most effective gene product at inducing synovial apoptosis in the rabbit model and also significantly inhibited inflammation through an unknown mechanism within 24 hours after injection. In contrast, P Tak (Amsterdam, The Netherlands) presented evidence that the identical p53 adenovirus was unable to confer a therapeutic effect in a rat model of arthritis. The reasons for this discrepancy between the effect of Ad-p53 in rabbit and rat models is unclear, but may represent the timing of injection or the level of infection of synovium.

Bone healing

Several investigators presented the use of gene transfer to accelerate bone healing. The enhancement of the bone healing process by transient, local gene delivery represents an attractive, achievable and appropriate application of gene therapy. A Baltzer (Dusseldorf, Germany) presented evidence that adenovirus-mediated delivery of bone morphogenetic protein (BMP)-2 and transforming growth factor (TGF)- β_1 directly into a critically sized segmental bone defect in rabbit femora led to complete bony union within 12 weeks. In contrast to the *in vivo* delivery method, D Gazat (Hebrew University, Jerusalem) described the use of *ex vivo* administration of MSCs, genetically modified to express BMP-2, to facilitate bone repair. Scaffolds to assist in the delivery of the genetically modified MSCs into the bone defects are being developed and tested.

The ability to prevent bone loss in a murine model of osteoporosis by systemic delivery of IL-1Ra by adenoviral infection also was presented (A Baltzer). Using a similar approach, D Bolon (Amgen, USA) demonstrated that systemic expression of the soluble decoy receptor osteoprotegerin (OPG) resulted in significantly more bone volume and fewer osteoclasts in ovariectomized mice. Interestingly, the systemic expression of OPG persisted for an extended period of time following intravenous injection of the Ad-OPG vector into mice, suggesting that OPG might also have effects on the immune response to virally infected cells.

Cartilage repair

M Brittberg (Goteborg University, Sweden) presented the results of a clinical trial using nongenetically modified chondrocytes that demonstrated the feasibility of performing autologous transplantation. Sixty-one patients were followed for a mean of 7.4 years, with 50 patients showing clinical improvement. Cartilage histology of the repair could be obtained with a second-look arthroscopy, and in 8 out of 12 cases, hyaline cartilage was observed. P Hernigou (Creteil, France) presented clinical data in hip osteonecrosis, where bone marrow cells containing MSCs were injected into the femoral head. Using the experimental SCID/hu model, D Gazat and D Noel (Montpellier, France) presented data on injection of engineered MSCs expressing BMP-2 under the control of a Tet-regulated promoter that resulted in cartilage formation in the knee joint. G Gross (Braunschweig, Germany) and C Jorgensen presented evidence that MSCs can be induced to differentiate into cartilage if modified to express BMP-2, cartilage-derived morphogenetic protein (CDMP)-1 and 2, Sox-9 or the T-box family of transcription factors. Cartilage repair was observed, but limited by endochondral bone formation after 4 weeks follow-up. The increase in angiogenesis observed after BMP-2 expression might be involved in the osteogenesis.

Matrix is critical for appropriate three-dimensional culture of progenitor cells, and for maintaining their chondral differentiation. A Facchini (Bologna, Italy) reported the use of a hyaluronic acid matrix as a support for the growth of immortalized chondrocytes that produced collagen II for the first 4 weeks. The future of cartilage repair may be the development of hybrid cartilage combining the matrix, the genes encoding growth factors and the progenitor cells.

Gene therapy to facilitate cartilage repair has been limited by the inability to deliver therapeutic genes directed to cartilage *in vivo*. As presented by S Ghivizzani (Harvard Medical School, USA), intra-articular expression of certain chondrogenic factors by virus-mediated gene transfer to synovium is one way to obviate this problem. Transfer of insulin-like growth factor (IGF)-1 and BMP-2 genes to the synovium in rabbits' knee joints increased matrix synthesis by the adjacent articular cartilage. Transfer of the TGF- β_1 gene in this way, however, led to severe adverse effects. Thus it appears advantageous to deliver the therapeutic genes directly into the cartilage defect, and Ghivizzani discussed various approaches to achieving this.

Clinical trials

The meeting also highlighted the current status of clinical trials for arthritis and related disorders. C Evans (Harvard Medical School, USA) presented the results of the first 'gene therapy for arthritis' clinical trial. The Phase I trial was designed to demonstrate safety and feasibility of transferring the gene for IL-1Ra to knuckle (MCP) joints in a three-dose escalation study enrolling nine patients. The *ex vivo* gene transfer of IL-1Ra was well tolerated in all nine patients treated, with evidence of intra-articular gene expression. P Wehling (Dusseldorf, Germany) presented similar results from three patients treated in an ongoing trial in Dusseldorf. There was a lively discussion regarding the ethical considerations regarding attempting clinical trials of a gene therapy for a nonlethal disease. Evans made a compelling argument for the Phase I trial in that it was based on extensive preclinical and safety efficacy data, and was reviewed and approved by an Institutional Review Board, an Institutional Biosafety Committee, the Recombinant DNA Advisory Committee of the National Institutes of Health, the United States Food and Drug Administration, the Director of the National Institutes of Health, and an External Review Board. Moreover, the positive results from the Phase I study should engender Phase II trials to evaluate efficacy of intra-articular gene transfer.

Future directions

The Second International Meeting on Gene and Cell Therapies of Arthritis and Related Disorders provided considerable optimism for the future. Clearly, gene therapy for joint and bone disorders is feasible, safe and effective in animal models. Moreover, it appears to be safe in a recently completed Phase I study. What is still needed is

the development of better vectors for direct gene transfer *in vivo* that allow for repeat dosing and long-term, regulated gene expression. In addition, although stem cell biology is progressing at a rapid rate, significantly more details of the signaling events that regulate differentiation of stem cells into bone or cartilage are needed. Finally, following appropriate rigorous safety testing and review, it will be important to start moving some of the well developed, effective preclinical approaches into the clinic. The field of gene therapy for joint and bone diseases is clearly moving forward and we believe there is a high likelihood that exciting progress will be presented at the Third International Meeting on Gene and Cell Therapies of Arthritis and Related Disorders, which is tentatively scheduled for the Spring of 2003.