Research article

Adverse effects of adenovirus-mediated gene transfer of human transforming growth factor beta 1 into rabbit knees

Zhibao Mi[1], Steven C Ghivizzani[1,3], Eric Lechman[1], Joseph C Glorioso[1], Christopher H Evans[2,3] and Paul D Robbins[1]

[1]Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA
[2]Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA
[3]Present address: Center for Molecular Orthopaedics, Harvard Medical School, Boston, Massacuhsetts, USA

Corresponding author: Paul D Robbins (e-mail: probb@pitt.edu)

Abstract

To examine the effect of transforming growth factor (TGF)-β1 on the regulation of cartilage synthesis and other articular pathologies, we used adenovirus-mediated intra-articular gene transfer of TGF-β1 to both naïve and arthritic rabbit knee joints. Increasing doses of adenoviral vector expressing TGF-β1 were injected into normal and antigen-induced arthritis rabbit knee joints through the patellar tendon, with the same doses of an adenoviral vector expressing luciferase injected into the contralateral knees as the control. Intra-articular injection of adenoviral vector expressing TGF-β1 into the rabbit knee resulted in dose-dependent TGF-β1 expression in the synovial fluid. Intra-articular TGF-β1 expression in both naïve and arthritic rabbit knee joints resulted in significant pathological changes in the rabbit knee as well as in adjacent muscle tissue. The observed changes induced by elevated TGF-β1 included inhibition of white blood cell infiltration, stimulation of glycosaminoglycan release and nitric oxide production, and induction of fibrogenesis and muscle edema. In addition, induction of chondrogenesis within the synovial lining was observed. These results suggest that even though TGF-β1 may have anti-inflammatory properties, it is unable to stimulate repair of damaged cartilage, even stimulating cartilage degradation. Gene transfer of TGF-β1 to the synovium is thus not suitable for treating intra-articular pathologies.

Keywords: arthritis gene therapy, cartilage degradation, inflammatory, nitric oxide, rabbit model, transforming growth factor-β1

Introduction

Transforming growth factor (TGF)-β is a dimeric protein of 25 kDa molecular weight, originally isolated from platelets [1,2]. There are three distinct mammalian isoforms, TGF-β1, TGF-β2 and TGF-β3, with TGF-β1 being the most abundant isoform. Almost all cell types express TGF-β, but the highest level of expression of TGF-β is in platelets and bone [3]. Mature TGF-β1 consists of two identical peptide chains, each containing 112 amino acids, linked via nine disulfide bonds [4]. TGF-β1 is synthesized as part of a large, latent protein complex, unable to bind to cellular receptors, with mature active TGF-β1 produced by cleavage [5].

TGF-β1 is a mutifunctional cytokine that plays an important role in immunomodulation, inflammation and tissue repair [6]. Many studies have suggested that TGF-β could be a potential therapeutic reagent for the repair of soft tissue and bone, and following ischemic injury. It may also have applications for the treatment of chronic inflammatory fibrotic and autoimmune diseases [7,8]. In contrast, other studies have demonstrated that TGF-β1 can cause inflammation and fibrosis [9,10]. The potential use of TGF-β1 for the treatment of human disease thus remains controversial [11].

Rheumatoid arthritis is a systemic, autoimmune disease. It is characterized by a chronic, erosive inflammation of painful and debilitating joints, with progressive degradation of cartilage and bone accompanied by proliferation of the synovium [12]. Rheumatoid arthritis remains incurable and, in many patients, difficult to treat. As a novel

approach to therapy, we and other workers have focused on developing the methods for local transfer of genes encoding therapeutic agents to the joint [13–19]. This strategy also can be applied to the treatment of osteoarthritis and for aiding the repair of the cartilage and other intra-articular tissues.

Since TGF-β1 has anti-inflammatory properties as well as being able to stimulate new matrix synthesis by chondrocytes, it represents a possible therapeutic agent with which to treat pathologies associated with rheumatoid arthritis and osteoarthritis by local gene delivery. Other workers and ourselves have previously examined the effects of TGF-β1 gene transfer on matrix synthesis in chondrocyte cultures, demonstrating a significant stimulation in the production of proteoglycans [9]. In addition, we have demonstrated that the TGF-β1 gene was able to overcome the inhibitory effects of IL-1β on matrix metabolism in chondrocytes in culture [20].

To examine the effect of TGF-β1 on joint pathology, we used adenovirus-mediated intra-articular gene delivery to confer sustained, intra-articular TGF-β1 expression in both naïve and arthritic rabbit knee joints. Intra-articular injection of adenoviral vector expressing human transforming growth factor (Ad.TGF)-β1 resulted in a high level of TGF-β1 accumulation in the synovial fluid. Intra-articular TGF-β1 expression was anti-inflammatory, inhibiting white blood cells. However, TGF-β1 expression also induced significant pathology in the rabbit knee as well as in the adjacent muscle, including stimulation of glycosaminoglycan (GAG) release and nitric oxide synthesis, and enhancement of fibrogenesis and muscle edema. These results suggest that, although TGF-β1 may have anti-inflammatory effects, sustained expression of TGF-β1 has adverse effects on joint pathology.

Materials and methods
Vector construction
The recombinant adenoviral vector used in the present study originates from replication-deficient type 5 adenovirus lacking E1 and E3 loci [21]. The human TGF-β1 cDNA was inserted in place of the E1 region in the shuttle plasmid pAd-Lox [22], where expression is driven by the cytomegalovirus promoter.

The recombinant Ad.TGF-β1 virus was generated by Cre-Lox-driven recombination in Cre 8 cells [22]. Briefly, a confluent $10 \, cm^2$ dish of Cre 8 cells (1.6×10^7) was split into five $6 \, cm^2$ dishes. Transfection of these cells with pAd-Lox-human TGF-β1 was performed by the calcium phosphate precipitation method with 3 μg pAd-Lox-human TGF-β1 construct digested with *Sfi*I and 3 μg ψ5 helper virus DNA. The transfected Cre 8 cells were fed daily until there were visible plaques. The cells were harvested and exposed to three cycles of freeze/thaw. The recombinant

virus was purified and amplified by infecting two 10 cm dishes of Cre 8 cells using 100 μl lysate. The Ad.TGF-β1 virus was purified using cesium chloride gradient ultracentrifugation at $154,000 \, g$ (30,000 rpm) and 4°C, and then dialyzed three times against Tris-buffered saline.

The titer was determined by measuring the viral DNA at optical density 260 nm ($OD_{260 \, nm}$) using the formula: viral particles = $OD_{260 \, nm} \times$ dilution / 9.09×10^{-13}. The adenovirus expressing luciferase was kindly provided by J Kolls (LSU Medical Center, New Orleans, LA, USA).

Animals and experimental arthritis
New Zealand white rabbits, weighing 4–5 kg, were purchased from Myrtles Rabbitry (Thompson Station, TN, USA). To establish antigen-induced arthritis (AIA), rabbits were sensitized to ovalbumin by intradermal injections of 5 mg ovalbumin emulsified in Freund's complete adjuvant. Arthritis was initiated in both hind knees of rabbits 3 weeks later by the intra-articular injection of 5 mg ovalbumin dissolved in 0.5 ml Gey's saline. The different adenoviral vectors were injected intra-articularly 24 hours after injection of antigen.

Experimental protocol
Twenty-four hours after induction of AIA, adenoviral particles encoding either the human TGF-β1 or luciferase were suspended in 0.2 ml Gey's saline and injected into the joint space of the knee through the patellar tendon. Different doses of virus (1×10^7, 1×10^8 and 1×10^9) viral particles were injected intra-articularly into three rabbits per group for analysis of the effects of TGF-β1 on naïve joint pathology, and the treated rabbits were sacrificed 7 days postinfection to observe the dose–response effects. Another group of three naïve rabbits was injected with 1×10^9 viral particles and sacrificed 17 days postinfection for long-term observation.

There were two groups of AIA rabbits used in the study. The first group of three rabbits was injected with 1×10^8 viral particles, and the second group of six rabbits was injected with 1×10^9 viral particles. Each rabbit received the indicated dose of TGF-β1 virus in one knee and the same amount of the adenoviral vector expressing luciferase (Ad.Luc) virus in the opposite knee as the control.

To lavage the rabbit knee joints, 1 ml Gey's saline was injected into the joint space through the patellar tendon. After manipulation of the joint, the needle was reinserted and the fluid was aspirated. Leukocytes in recovered lavage fluids were counted using a hemocytometer. The levels of TGF-β1 in conditioned media, lavage fluids and sera were measured using an ELISA kit (R & D Systems, Minneapolis, MN, USA) as directed by the supplier. The levels of sulfated GAGs in lavage fluids were determined using a colorimetric dye-binding assay using 1,9-dimethyl-

Figure 1
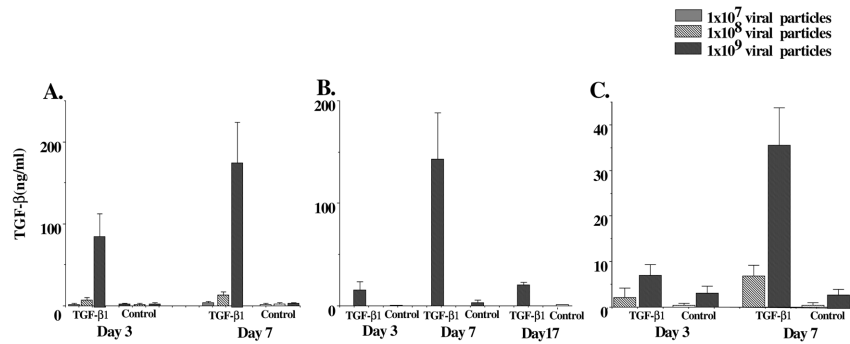
Adenovirus-mediated transforming growth factor (TGF)-β1 gene expression in naïve and antigen-induced arthritis (AIA) rabbit joints. **(A)** 1×10^7, 1×10^8 and 1×10^9 adenoviral particles encoding human TGF-β1 cDNA were injected into naïve rabbit left knees. **(B)** 1×10^9 viral particles were injected into naïve rabbit left knees. **(C)** 1×10^8 and 1×10^9 viral particles were injected into AIA rabbit left knees. The same amounts of control viral particles were injected into the contralateral knees. Levels of TGF-β1 are expressed in nanograms per milliliter of lavage fluid recovered from knees 3, 7 and 17 days postinfection. All values are expressed as mean ± standard error of the mean.

methylene blue [23]. The levels of total nitrite in lavage fluids were measured with Nitric Oxide Assay kits (Calbiochem®; Biosciences Inc, La Jolla, CA, USA).

Articular cartilage fragments shaved from the femoral condyles were placed into 1 ml Neuman–Tyell serum-free medium (Gibco, New York, USA) to measure the rate of proteoglycan synthesis. The fragments were then incubated with $^{35}SO_4^{2-}$ (20 µCi) for 24 hours at 37°C, and the media harvested and stored at −20°C. Proteoglycans were extracted from the cartilage by incubation for 48 hours in 1 ml of 0.5 M NaOH at 4°C with gentle agitation. Following chromatographic separation of unincorporated $^{35}SO_4^{2-}$ using PD-10 columns (Pharmacia, Uppsala, Sweden), the levels of radiolabeled GAGs released onto the culture media or recovered by alkaline extraction were quantitated by scintillation counting [24].

Histology
For histological analyses, tissues harvested from dissected knees were first fixed in 10% formalin. The fixed tissues were imbedded in paraffin, sectioned at 5 µm, and stained with H & E.

Statistical analysis
All data collected are expressed as mean ± standard error. Statistical significance was analyzed by analysis of variance and Student's *t* test. Correlation coefficients (*r*) were calculated using Pearson's method.

Results
Expression of TGF-β1 after intra-articular injection of Ad.TGF-β1
To test the effects of adenoviral-mediated human TGF-β1 gene expression in naïve and AIA rabbit joints, 1×10^7,

1×10^8 and 1×10^9 particles of Ad.TGF-β1 were injected into either naïve or arthritic rabbit knees. The same amounts of Ad.Luc were injected into the contralateral knees. Lavages were performed on day 3, day 7 and day 17, and the levels of TGF-β1 were determined by ELISA (Fig. 1).

TGF-β1 expression was detected in all knee joints receiving either 1×10^8 or 1×10^9 viral particles, with higher levels detected in the arthritic knees. A significant drop in TGF-β1 expression was observed after 17 days of post-viral injection. No significant levels of TGF-β1 were detected in the 1×10^7 viral particle injection group and in the contralateral joints receiving the different doses of luciferase virus. Furthermore, no significant expression of TGF-β was detected in the serum.

It is important to note that detection of TGF-β1 in the synovial fluid required acid activation, suggesting that the protein is in its latent form. Moreover, there were no observed therapeutic or adverse effects following intra-articular injection of the low dose (1×10^7 particles) of Ad.TGF-β1.

Alterations in joint anatomy after intra-articular Ad.TGF-β1 injection
Three days after injection of Ad.TGF-β1, the knees receiving the highest dose of virus became enlarged with a reduction in joint movement. In addition, the muscles adjacent to the joints showed signs of swelling and reduced movement. The animals were sacrificed on day 7 post-injection, and the joints were analyzed. The size of the joints and the adjacent muscles increased dramatically both in naïve rabbits (1.5 × contralateral knees, $P < 0.05$) and in AIA rabbits (1.25 × contralateral knees, $P < 0.01$) (Fig. 2).

Figure 2
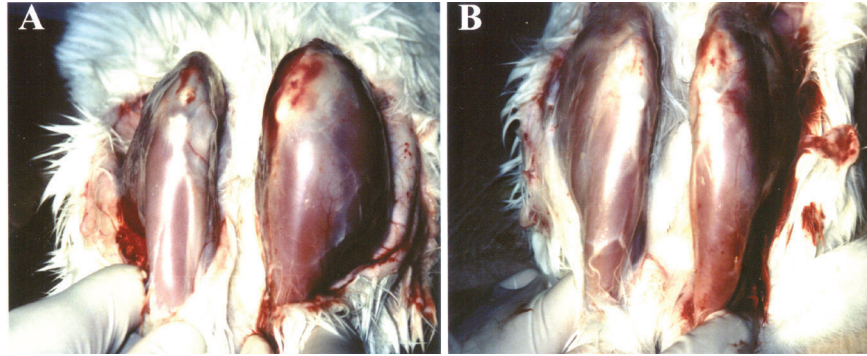
Gross pathology caused by intra-articular injection of adenoviral vector expressing human transforming growth factor beta 1 (Ad.TGF-β1) virus. **(A)** The naïve rabbit left knee injected with a high dose of the TGF-β1 virus and the right knee injected with a high dose of the luciferase virus. **(B)** The naïve rabbit left knee with a low dose of the Ad.TGF-β1 virus and the right knee with a low dose of the luciferase virus. These images were taken when the rabbits were sacrificed on day 7.

The movement of joints was also severely limited, with the Ad.Luc contralateral knees moving freely at 180° whereas the knees treated with Ad.TGF-β1 virus could only move at 90–120°. The limitation to joint movement was not due to the enlarged muscles since, when the muscles were cut away, the limitation of movement was still observed. In addition, when the joints were analyzed 17 days after viral injection, at a time when the muscle size returned to normal, the joint still could not move freely. The limitation to joint movement could thus be due to possible synovial hyperplasia or effects on ligaments or cartilage metabolism.

It is important to note that we did observe an increase in creatine kinase levels in the serum that would suggest muscle damage (data not shown). In contrast to the high-dose TGF-β1 group, only a very mild effect was observed on the gross joint structure in the group receiving 1×10^8 viruses and no changes were observed in the group receiving 1×10^7 viruses.

Effect of TGF-β1 on cartilage metabolism
To determine whether overexpression of TGF-β1 had effects on cartilage metabolism in naïve and AIA rabbit joints, GAG synthesis by articular cartilage and GAGs released into synovial fluid as a result of proteoglycan breakdown were measured. The rabbit joints receiving Ad.TGF-β1 had significant higher levels of GAG release, compared with the contralateral Ad.Luc joints, in lavage fluids at day 3, day 7 and day 17 for the naïve rabbits and at day 7 for the AIA rabbits (Fig. 3A–C). GAG release levels correlated linearly with the levels of TGF-β1 in lavage fluids ($r=0.937$) in the naïve rabbits. In addition, only the highest dose of Ad.TGF-β1 was able to stimulate GAG synthesis in the naïve rabbit joints from day 7 and day 17, but the stimulation was marginal. There was no statistically significant difference between GAG synthesis

by the naïve rabbits with the two lower doses of viral injections and in the AIA rabbits (Fig. 3D–F).

Taken together, these results suggest that intra-articular expression of TGF-β1 stimulated cartilage matrix degradation while having only a minor effect on the promotion of new matrix synthesis. This is in contrast to the results observed on matrix synthesis in chondrocytes in culture, where TGF-β1 was able to stimulate significant new matrix synthesis as well as overcome the suppressive effects of IL-1β on matrix metabolism [21,25].

Inhibition of white blood cell infiltration and elevation of nitric oxide synthesis
To determine whether TGF-β1 expression could inhibit the mild inflammation induced by intra-articular injection of high doses of adenovirus or the severe inflammation occurring in the AIA model, the levels of white blood leukocytic infiltrate in the synovial lavage fluids were determined (Fig. 4).

The joints of naïve rabbits receiving the highest dose of Ad.TGF-β1 adenovirus had significantly lower levels of white blood cell infiltration in lavage fluids at day 3, day 7 and day 17. The white blood cell infiltration in the naïve joints directly correlated with TGF-β1 expression levels in the lavage fluids ($r=0.954$). In the AIA rabbit knee joints, there was a reduction in the infiltration at day 3 and day 7 compared with the contralateral control Ad.Luc joints, consistent with TGF-β1 having an anti-inflammatory effect. Surprisingly, TGF-β1 expression elevated nitrate levels in the joints receiving high-dose injections of TGF-β1 adenovirus at day 3, day 7 and day 17 for naïve rabbits and at day 7 for the AIA rabbits, compared with the control joints (Fig. 4D–F). The nitrate levels also directly correlated with the levels of TGF-β1 in lavage fluids ($r=0.945$) for naïve rabbits.

Figure 3
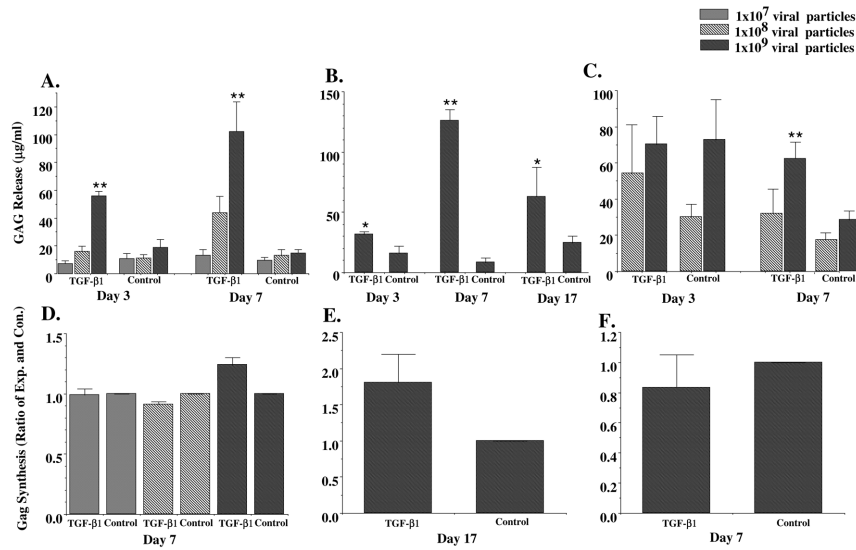
Glycosaminoglycan (GAG) release into lavage fluids and GAG synthesis by articular cartilage recovered from the rabbit knees injected with adenoviral vector expressing human transforming growth factor beta 1 (Ad.TGF-β1) (Exp.) or the control adenoviruses (Con.). **(A, D)** Short-term naïve rabbits. **(B, E)** Long-term naïve rabbits. **(C, F)** Antigen-induced arthritis rabbits. All values are expressed as the mean ± standard error of the mean. * $P < 0.05$ and ** $P < 0.01$, compared with contralateral knees.

Figure 4
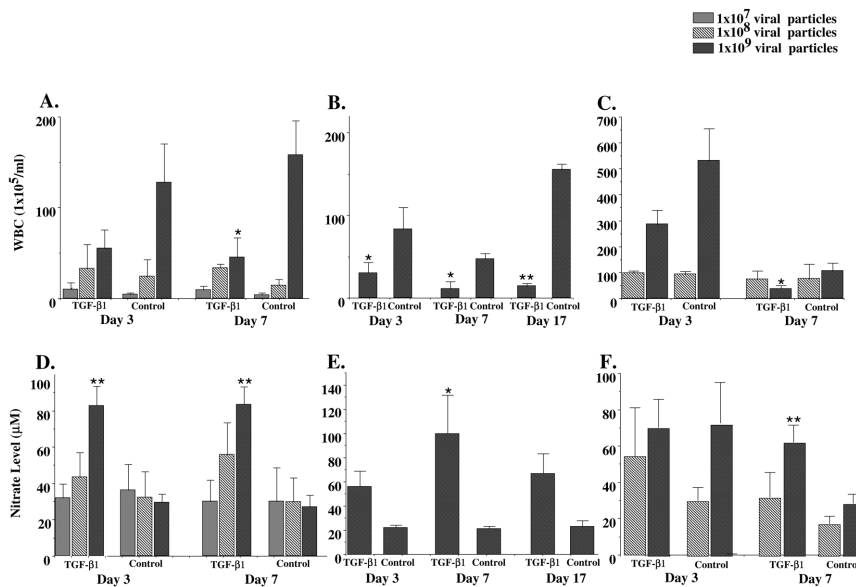
White blood cell (WBC) infiltration and nitrate levels in lavage fluids recovered from the rabbit knees injected with adenoviral vector expressing human transforming growth factor beta 1 (Ad.TGF-β1) or the control adenoviruses. **(A, D)** Short-term naïve rabbits. **(B, E)** Long-term naïve rabbits. **(C, F)** Antigen-induced arthritis rabbits. All values are expressed as the mean ± standard error of the mean. * $P < 0.05$ and ** $P < 0.01$, compared with contralateral knees.

These results suggested that TGF-β1 is indeed anti-inflammatory in arthritic knees, but is able to induce production of nitric oxide through an unknown mechanism.

Histological analysis of the intra-articular effects of TGF-β1

The naïve and AIA rabbit knee joints receiving Ad.TGF-β1 and Ad.Luc were also examined by histology. There was

Figure 5
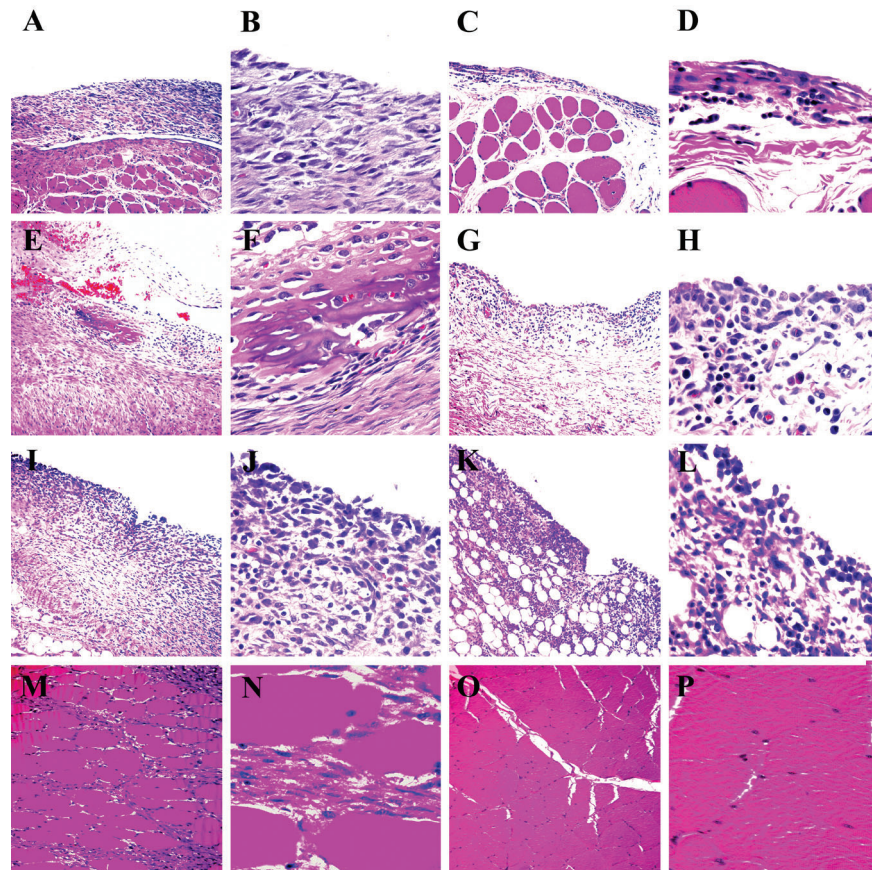
Histological analyses of synovial tissue recovered from rabbit knees joints and muscular tissue adjacent to the joints. (**A, B**) Naïve rabbit left knees were injected with 1×10^9 adenoviral vector expressing human transforming growth factor beta 1 (Ad.TGF-β1) viral particles. (**C, D**) The contralateral knee joint of (A, B). (**E, F**) Long-term naïve rabbit left knees were injected with 1×10^9 Ad.TGF-β1 viral particles. (**G, H**) The contralateral knee joint of (E, F). (**I, J**) Antigen-induced arthritis rabbit knees were injected with 1×10^9 Ad.TGF-β1 viral particles. (**K, L**) The contralateral knee of (I, J). (**M, N**) Muscle from an adjacent naïve rabbit knee with 1×10^9 Ad.TGF-β1 viral particles. (**O, P**) The contralateral knee of (M, N). (B), (F), (J), and (N) High magnification (400×) images of (A), (E), (I), and (M) (100×), respectively. (D), (H), (L), and (P) High magnification (400×) images of (C), (G), (K), and (O) (100×), respectively.

significant fibroblast proliferation around the myofibers in the naïve rabbits joint receiving Ad.TGF-β1. There was also mild hyperplasia of the synovial lining, but without any evidence of inflammatory cells being observed (Fig. 5A,B). There were some inflammatory cells in the contralateral synovial lining, but no evidence of synovitis (Fig. 5C,D). The synovium from the TGF-β1 virus treated joints was highly fibrotic 17 days after viral injection, with evidence of osteometroplasia found in the synovium (Fig. 5E,F) but with no evidence of inflammation or angiogenesis (Fig. 5F).

There was mild inflammation under the synovium in the contralateral joints receiving Ad.Luc (Fig. 5G,H). The synovium in the Ad.TGF-β1-treated AIA rabbits showed evidence of hyperproliferation with mild inflammation (Fig. 5I,J), compared with the contralateral control joints that had severe inflammation (Fig. 5K,L). In the muscle tissue adjacent to Ad.TGF-β1-treated joints, there was evi-

dence of both fibroblast and myofibroblast proliferation between myofibers with intracellular edema, but there was no evidence of inflammation or myonecrosis (Fig. 5M,N). In contrast, the muscle tissue from the contralateral controls knees was normal (Fig. 5O,P). There was also mild fibroblast proliferation and synovial inflammation in the 1×10^8 Ad.TGF-β group, but no significant histological changes were observed in the 1×10^7 viral group.

These data taken together suggest that TGF-β1 is able to stimulate fibrogenesis and to suppress inflammation. Moreover, the results suggest that elevated TGF-β1 levels result in chondrogenesis within the synovial tissue.

Discussion

TGF-β1 is a mutifunctional cytokine that plays an important role in immunomodulation, inflammation and tissue repair. Given that TGF-β1 is able to induce new matrix

synthesis from chondrocytes in culture as well as able to block inflammation *in vivo*, it has been proposed that local intra-articular gene transfer of TGF-β1 could be therapeutic for the treatment of rheumatoid arthritis as well as osteoarthritis. To examine the effects of TGF-β1 on joint pathology, we used adenovirus-mediated intra-articular gene delivery to confer sustained intra-articular TGF-β1 expression in both naïve and arthritic rabbit knee joints. Intra-articular injection of Ad.TGF-β1 virus into the rabbit knee resulted in dose-dependent elevated levels of expression of TGF-β1 in the synovial fluid, but not in the serum.

Intra-articular TGF-β1 expression resulted in dose-dependent biological effects in the rabbit knee as well as in adjacent muscle. In particular, local intra-articular expression in naïve joints stimulated cartilage breakdown, as measured by synovial GAG levels, without enhancing new matrix synthesis. In addition, TGF-β1 expression stimulated nitric oxide production. Similarly, in arthritic joints where TGF-β1 expression inhibited white blood cell infiltration, it also stimulated GAG release and nitric oxide production. Although there was a reduction in inflammation in arthritic joints, TGF-β1 expression induced fibrogenesis and muscle edema. In addition, TGF-β1 expression in the adenovirally infected synovial lining also resulted in induction of chondrogenesis in the synovium. Elevated TGF-β1 expression in the synovial fluid thus resulted in a variety of adverse pathological changes.

A previous study examined the effect of Ad.TGF-β1 in the knee joints of naïve C57/Bl/6 mice where gene transfer of TGF-β1 to the mouse knee resulted in hyperplasia of the synovium as well as in chondro-osteophyte formation at the chondrosynovial junctions [10]. The present experiments have shown similar effects on synovial proliferation, but also have extended the murine studies to examine the effects of TGF-β1 in both diseased knee joints and normal knee joints in the rabbit. Similar to the previous report, we have observed evidence for intra-synovial chondrogenesis as well as osteometroplasia following TGF-β1 gene transfer. Whether TGF-β1 directly or indirectly stimulates proliferation of synovium is unclear, but this pathology apparently is mediated through an IL-1β-independent mechanism.

It has been speculated that intra-articular delivery of TGF-β1 would result in enhanced synthesis of new matrix [9]. Indeed, we have reported previously that the TGF-β1 gene is more effective than insulin-like growth factor type 1 and bone morphogenetic protein type 2 genes in stimulating new matrix synthesis from rabbit chondrocytes in culture. We have also demonstrated that TGF-β1 is able to partially overcome the inhibition of new matrix synthesis by IL-1β in cultured chondrocytes. The results presented in the present article, however, suggest that TGF-β1 expression is unable to enhance new matrix synthesis *in vivo* in either naïve joints or, in particular, in diseased

joints. Moreover, it appears as if TGF-β1 confers adverse effects by stimulating cartilage degradation through an unknown mechanism. In contrast to the adverse effects of intra-articular adenoviral gene transfer of TGF-β1, we have shown that gene transfer of insulin-like growth factor type 1 to the rabbit knee results in an increase in new matrix synthesis without any adverse effects [17].

Taken together, these results suggest that increasing the intra-articular levels of TGF-β1 has no therapeutic effect on cartilage metabolism, resulting instead in higher rates in cartilage degradation. Use of the synovium as a target tissue for TGF-β1 gene transfer, resulting in elevating the intra-articular level, is thus not appropriate for the enhancement of repair of cartilage defects. Instead, for TGF-β1 gene therapy to be effective in promoting repair of damaged cartilage, the level of TGF-β1 will need to be highly regulated as well as expression localized. TGF-β1 expression would need to be targeted, at the appropriate levels, to the site of cartilage damage, such as through gene transfer to chondrocytes or stem cells involved in repairing the damaged tissues.

TGF-β1 has been shown to be therapeutic in several different animal models when expressed systemically from muscle tissue [26,27]. This suggests that elevated serum levels of TGF-β1 can reduce general inflammation as well as inhibit IL-1β and tumor necrosis factor alpha production, resulting in a systemic therapeutic effect. In addition, TGF-β1 has been shown to be therapeutic in murine models of collagen-induced arthritis following delivery in genetically modified T cells [28]. This observation suggests that targeting TGF-β1 to certain sites of inflammation through the use of arthogenic T cells also can be therapeutic. However, our results suggest that local expression of TGF-β1, unlike systemic expression, is not therapeutic due to adverse pathologies associated with elevated intra-articular TGF-β1 expression.

Although our results do not preclude the development of gene therapy approaches to express regulated TGF-β1 systemically to downmodulate the immune response, the results suggest that any clinical application of local TGF-β1 gene transfer should proceed with caution. TGF-β1 is clearly a potent cytokine, able to confer multiple effects when expressed intra-articularly *in vivo*.

Conclusion
Gene transfer represents a novel method for obtaining high intra-articular levels of therapeutic agents for the treatment of arthritis. TGF-β1 is able to stimulate new matrix synthesis by chondrocytes in culture as well as able to reduce inflammation *in vivo*. In this report, the effects of intra-articular expression on both naïve and arthritic rabbit knee joint pathology were examined by adenoviral-mediated intra-articular gene transfer of TGF-β1. The results

suggest that elevated TGF-β1 expression confers adverse joint pathology including an increase in cartilage matrix degradation without stimulating new matrix synthesis. In addition, TGF-β expression induces muscle edema and fibrogenesis.

Although TGF-β1 may have certain anti-inflammatory properties, it is unable to stimulate repair of damaged cartilage, and even stimulates cartilage degradation. Moreover, elevated TGF-β1 expression also induced muscle edema and fibrogenesis. Gene transfer of TGF-β1 to the synovium is thus not suitable for treating intra-articular pathologies, such as the repair of damaged cartilage, associated with rheumatoid arthritis and osteoarthritis.

Competing interests
None declared.

Acknowledgments

References
1. Sporn MB, Roberts AB: **TGF-beta: problems and prospects.** *Cell Regulat* 1990, **1**:875-882.
2. Roberts AB, Sporn MB: **Physiological actions and clinical applications of transforming growth factor-beta (TGF-beta).** *Growth Factors* 1993, **8**:1-9.
3. Assoian RK, Komoriya A, Meyers CA, Miller DM, Sporn MB: **Transforming growth factor-beta in human platelets. Identification of a major storage site, purification, and characterization.** *J Biol Chem* 1983, **258**:7155-7160.
4. Sharples K, Plowman GD, Rose TM, Twardzik DR, Purchio AF: **Cloning and sequence analysis of simian transforming growth factor-beta cDNA.** *DNA* 1987, **6**:239-244.
5. Miller DA, Pelton RW, Derynck R, Moses HL: **Transforming growth factor-beta. A family of growth regulatory peptides.** *Ann NY Acad Sci* 1990, **593**:208-217.
6. Sporn MB, Roberts AB: **Transforming growth factor-beta. Multiple actions and potential clinical applications.** *JAMA* 1989, **262**:938-941.
7. Beck SL, Chen TL, Mikalauski P, Ammann AJ: **Recombinant human transforming growth factor beta 1 (rhTGF-β1) enhances healing and strength of granulation skin wounds.** *Growth Factors* 1990, **3**:267-275.
8. Prud'homme GJ, Piccirillo CA: **The inhibitory effects of transforming growth factor-beta-1 (TGF-beta1) in autoimmune diseases.** *J Autoimmun* 2000, **14**:23-42.
9. Shuler FD, Georgescu HI, Niyibizi C, Studer RK, Mi Z, Johnstone B, Robbins RD, Evans CH: **Increased matrix synthesis following adenoviral transfer of a transforming growth factor beta1 gene into articular chondrocytes.** *J Orthop Res* 2000, **18**:585-592.
10. Bakker AC, van de Loo FA, van Beuningen HM, Sime P, van Lent PL, van der Kraan PM, Richards CD, van den Berg WB: **Overexpression of active TGF-beta-1 in the murine knee joint: evidence for synovial-layer-dependent chondro-osteophyte formation.** *Osteoarthritis Cartilage* 2000, **9**:128-136.
11. Evans CH, Ghivizzani SC, Lechman ER, Mi Z, Jaffurs D, Robbins PD: **Lessons learned from gene transfer approaches.** *Arthritis Res* 1999, **1**:21-24.
12. Evans C, Robbins PD: **Prospects for treating arthritis by gene therapy.** *J Rheumatol* 1994, **21**:779-782.
13. Hung GL, Galea-Lauri J, Mueller GM, Georgescu HI, Larkin LA, Suchanek MK, Tindal MH, Robbins PD, Evans CH: **Suppression of intra-articular responses to interleukin-1 by transfer of the interleukin-1 receptor antagonist gene to synovium.** *Gene Ther* 1994, **1**:64-69.
14. Bandara G, Mueller GM, Galea-Lauri J, Tindal MH, Georgescu HI, Suchanek MK, Hung GL, Glorioso JC, Robbins PD, Evans CH: **Intraarticular expression of biologically active interleukin 1-receptor-antagonist protein by ex vivo gene transfer.** *Proc Natl Acad Sci USA* 1993, **90**:10764-10768.
15. Watanabe S, Imagawa T, Boivin GP, Gao G, Wilson JM, Hirsch R: **Adeno-associated virus mediates long-term gene transfer and delivery of chondroprotective IL-4 to murine synovium.** *Mol Ther* 2000, **2**:147-152.
16. Ghivizzani SC, Lechman ER, Tio C, Mule KM, Chada S, McCormack JE, Evans CH, Robbins PD: **Direct retrovirus-mediated gene transfer to the synovium of the rabbit knee: implications for arthritis gene therapy.** *Gene Ther* 1997, **4**:977-982.
17. Mi Z, Ghivizzani SC, Lechman ER, Jaffurs D, Glorioso JC, Evans CH, Robbins PD: **Adenovirus-mediated gene transfer of insulin-like growth factor 1 stimulates proteoglycan synthesis in rabbit joints.** *Arthritis Rheum* 2000, **43**:2563-2570.
18. Quattrocchi E, Dallman MJ, Feldmann M: **Adenovirus-mediated gene transfer of CTLA-4Ig fusion protein in the suppression of experimental autoimmune arthritis.** *Arthritis Rheum* 2000, **43**:1688-1697.
19. Lechman ER, Jaffurs D, Ghivizzani SC, Gambotto A, Kovesdi I, Mi Z, Evans CH, Robbins PD: **Direct adenoviral gene transfer of viral IL-10 to rabbit knees with experimental arthritis ameliorates disease in both injected and contralateral control knees.** *J Immunol* 1999, **163**:2202-2208.
20. Smith P, Shuler FD, Georgescu HI, Ghivizzani SC, Johnstone B, Niyibizi C, Robbins PD, Evans CH: **Genetic enhancement of matrix synthesis by articular chondrocytes: comparison of different growth factor genes in the presence and absence of interleukin-1.** *Arthritis Rheum* 2000, **43**:1156-1164.
21. Yeh P, Perricaudet M: **Advances in adenoviral vectors: from genetic engineering to their biology.** *FASEB* 1997, **11**:615-623.
22. Hardy S, Kitamura M, Harris-Stansil T, Dai Y, Phipps ML: **Construction of adenovirus vectors through Cre-lox recombination.** *J Virol* 1997, **71**:1842-1849.
23. Farndale RW, Buttle DJ, Barnette AJ: **Improved quantitation and discrimination of sulfated glycosaminoglycans by use of dimethymethylene blue.** *Bioch Biophys Acta* 1986, **883**:173-177.
24. Taskiran D, Stefanovic-Racic M, Georgescu HI, Evans CH: **Nitric oxide mediates suppression of cartilage proteoglycan synthesis by interleukin-1.** *Biochem Biophys Res Commun* 1994, **200**:142-148.
25. Moller HD, Fu FH, Niyibizi C, Studer RK, Georgescu HJ, Robbins PD, Evans CH: **TGF-beta-1 gene transfer in joint cartilage cells. Stimulating effect in extracellular matrix synthesis.** *Orthopade* 2000, **29**:75-79
26. Song XY, Gu M, Jin WW, Klinman DM, Wahl SM: **Plasmid DNA encoding transforming growth factor-beta1 suppresses chronic disease in a streptococcal cell wall-induced arthritis model.** *J Clin Invest* 1998, **101**:2615-2621.
27. Song XY, Zeng L, Jin W, Pilo CM, Frank ME, Wahl SM: **Suppression of streptococcal cell wall-induced arthritis by human chorionic gonadotropin.** *Arthritis Rheum* 2000, **43**:2064-2072.
28. Chernajovsky Y, Adams G, Triantaphyllopoulos K, Ledda MF, Podhajcer OL: **Pathogenic lymphoid cells engineered to express TGF beta 1 ameliorate disease in a collagen-induced arthritis model.** *Gene Ther* 1997, **4**:553-559.

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