Gene Ther Mol Biol Vol 3, 243-248. August 1999.

Gene therapy for arthritis

Review Article

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Abbreviations: **RA**, rheumatoid arthritis; **CIA**, collagen-induced arthritis

Key words: Gene therapy, in vivo gene transfer, rheumatoid arthritis, inflammation, gene expression

Received: 10 October 1998; accepted: 15 October 1998

Summary

Gene therapy for the treatment of arthritis is developing rapidly. The ability to deliver genes to local sites of inflammation decreases the possibility of systemic side effects, making arthritis a good candidate for gene therapy. Animal models of arthritis provide a means of testing gene transfer strategies. Several issues still need to be addressed including which genes to deliver, how to deliver these genes, and how to regulate gene expression in vivo.

I. Introduction

Arthritis is a major health problem among working age people in the United States, with greater than 2 million men and greater than 3 million women reporting activity limitation (Yelin, 1992). The most prevalent form of arthritis, rheumatoid arthritis (RA), affects approximately 0.8% of most populations (Koopman, 1997). Arthritic symptoms have been reported for 55% of individuals ≥70 years of age. Among these elderly affected individuals, 3/4 were limited in physical actions and 1/3 were limited in daily living (Yelin, 1992). Therefore, the chronic symptoms of arthritis impact significantly on the quality of life.

Until recently, the treatment of arthritis, particularly RA, involved the use of non-specific anti-inflammatory agents, such as nonsteroidal anti-inflammatory agents (NSAIDs), steroids and methotrexate. These anti-rheumatic drugs allow relief of many symptoms of the disease, but can exhibit harmful side effects and do not necessarily alter the natural course of the disease (Koopman, 1997). The cause of RA is unknown, and the mechanism of action of many of the drugs used to treat RA remains unknown. However, recent studies of molecular and cellular mechanisms that govern the pathophysiology of arthritis has led to the discovery of therapeutic biological agents that offer greater specificity in the treatment of arthritis. These biological agents are currently being delivered primarily at the protein level. The short half-life of these molecules necessitates frequent re-administration. These naturally-produced molecules have the potential to be delivered via gene transfer, which may allow for a reduction in the requirement for frequent re-administration of the drug.

II. Gene delivery strategies

A. In vivo gene transfer

Although RA often affects local joints, immunological responses observed in patients with RA demonstrate the presence of systemic components of arthritis. Therefore, the treatment of RA can be approached with either systemic or local therapies.

Systemic gene delivery, such as by i.v. administration, has been demonstrated in animal models. These studies have examined mainly the short-term effects on arthritis and not the long-term systemic effects, including potential toxicity. Therefore, the delivery of genes directly into the bloodstream requires further investigation.

For the treatment of arthritis, local gene delivery is an attractive therapeutic option. Since the target of arthritis is the synovium or cells contained within the affected joint, local therapies involving injection directly into the affected joint space, could potentially provide delivery of genes to a limited space and reduce toxic systemic effects. Local

injection of adenovirus encoding a reporter gene to inflamed joints of monkeys with CIA results in expression that is contained to the synovium and is not present in other tissue samples, indicating that gene transfer to synovial tissue may be safe in primates and may exhibit an ideal biodistribution (Goossens, 1997). However, local administration of adenovirus in other animal models has effects on distal joints, suggesting that local delivery of gene products may produce systemic effects that must be analyzed appropriately (Bakker, 1997; Ghivizzani, 1998; May, 1998).

B. Ex vivo gene transfer

The ex vivo approach involves removal of synovial cells, culturing and infection of these cells with the appropriate virus, usually retrovirus, and subsequently returning the cells to the joint space. This procedure, while cumbersome and expensive, also provides for analysis and selection of the genetically altered cells before returning them to the joint space.

III. Gene transfer vectors

A. Viral vectors

Various gene therapy vectors have been utilized that can be grouped mainly into viral and non viral vectors. Since viruses naturally deliver genetic material to cells, the use of viruses is the basis for most gene delivery systems. Viruses are the most widely used means of delivering genes in arthritic animal models (Nita, 1996). Among viral vectors, retroviral and adenoviral vectors are primarily used for gene delivery, and both have particular characteristics that make them suitable for the delivery of genes in the treatment of arthritis.

Adenoviruses are easily produced at high titres and infect non dividing synovial cells. Adenovirus delivery of the β-galactosidase gene intra-articularly demonstrates that adenovirus can infect non dividing synovial cells and β-galactosidase expression can last up to 21 days (Sawchuck, 1996). Intravenous administration of adenovirus encoding vIL-10 also indicates that vIL-10 can be detected up to 7 days after injection and can inhibit CIA (Apparailly, 1998; Ma, 1998). However, adenoviral vectors induce an inflammatory response, which may come from the viral proteins being expressed or the transgene product itself. In terms of gene expression, adenoviral encoded proteins are normally short-lived, which is thought to be due to this inflammatory process.

Retroviruses are produced at relatively low titres, infect only dividing cells, and incorporate into the host genome. Retroviral vectors are primarily used ex vivo to transfect cultured synovial cells that divide, allowing for retroviral infection. Recent studies indicate that stimulation of cells with TNFα in vitro allows retroviral transduction of cells (Jorgensen, 1997), and that inflamed synovium, which produces TNFα, may be more susceptible to retroviral uptake (Ghivizzani, 1997). These findings suggest that retroviral vectors might be delivered intra-articularly to target inflamed synovium. Long term gene expression is desirable for any gene therapy vector. Incorporation of the retrovirus into the host genome allows for long term gene expression; however, with this incorporation the risk of insertional mutagenesis exists. Unlike adenovirus-infected cells, retrovirus-infected cells have not been a target for destruction by the immune system (Evans, 1997).

Another kind of viral vector, the lentivirus, is derived from retroviruses, but has the capability to infect non dividing cells (Naldini, 1996). This virus may have promise for targeting non dividing synovial cells in the treatment of arthritis.

B. Non viral vectors

Various methods of non viral gene delivery include liposomal delivery, direct plasmid injection, and gene gun delivery. Non viral DNA delivery offers low toxicity, but most methods available are very inefficient at transfection of synovial cells. Gene transfer to rabbit and rat synovial cells by direct plasmid injection demonstrates that plasmid uptake resembles non specific endocytosis (Yovandich, 1995). The transient expression of the reporter plasmid corresponds with the degradation of plasmid DNA, indicating that intra-articular injection of plasmid DNA results in short-term gene expression. Long term gene expression of non viral plasmid DNA vectors has been achieved in muscle tissue (Tripathy, 1996). Expression of certain genes in skeletal muscle via plasmid injection has systemic effects on the immune system (Raz, 1993). Plasmid DNA encoding TGF-β delivered into thigh muscle of rats with streptococcal cell wall induced arthritis, suppressed the chronic disease and virtually eliminated subsequent inflammation and arthritis (Song, 1998). Therefore, intra-muscular injection of plasmid may be a less toxic way to systemically deliver anti-inflammatory products for the treatment of arthritis.

IV. Candidate genes

Analysis of cytokine expression between arthritic and non-arthritic joints indicates an increase in a number of cytokines in arthritic joints. This information has led to two main strategies to reduce inflammation in arthritic joints. The first approach involves the use of natural inhibitors of pro-inflammatory cytokines. The second approach, immune deviation, involves administration of cytokines that naturally down regulate pro-inflammatory cytokine synthesis.

A. Natural inhibitors of inflammatory cytokines

TNF-α and IL-1 are major regulators of inflammation in arthritic joints. Inhibitors of these two cytokines reduce arthritis in both animal models of arthritis and in ongoing human trials. In collagen-induced arthritis (CIA), an animal model of RA, treatment with antibody to TNFα (Joosten, 1994; Thorbecke, 1992) or IL-1β (Geiger, 1993; Joosten, 1994; Joosten, 1996; Thorbecke, 1992) reduced disease severity. In human trials administration of cA2, an antibody specific for TNFα, dramatically suppressed symptoms of disease, although this effect required continual treatment (Elliott, 1994; Elliott, 1994; Elliott, 1993).

1. Interleukin-1 receptor antagonist (IL-1Ra)

IL-1Ra regulates IL-1 activity in vivo by binding to IL-1 receptors. IL-1Ra, while inhibiting IL-1 from binding, itself does not stimulate activity through the IL-1 receptor. However, a 10-100 fold excess of IL-1Ra over IL-1 is necessary to block the effects of IL-1 activity in vivo (Dinarello, 1991; Hirsch, 1996). Continuous administration of high levels of IL-1Ra can block CIA (Joosten, 1996; Wooley, 1993). Transgenic mice overproducing IL-1Ra exhibit a reduction in the incidence and severity of CIA, and mice lacking IL-1Ra have a significantly earlier onset of CIA (Ma, 1998). Human trials aimed at determining the efficacy of administration of recombinant human IL-1Ra are still being assessed (Campion, 1996). These studies indicate that IL-1Ra is a good candidate gene for reduction of arthritis.

Several animal models of arthritis have shown benefits after IL-1Ra gene delivery. Expression of human IL-1Ra in rabbits with antigen induced arthritis changed the course of arthritis and suppressed the effects of IL-1 (Bandara, 1993; Otani, 1996). Ex vivo retroviral transduction of primary synoviocytes grafted to ankle joints in rats with bacterial cell wall-induced arthritis showed a significantly suppressed severity of recurrence of arthritis and attenuated erosion of cartilage and bone (Makarov, 1996). Treatment of mice with CIA by ex vivo transduction of NIH/3T3 fibroblasts with retrovirus expressing human IL-1Ra prevented CIA in injected knee joints and the "draining" paws (Bakker, 1997). Rabbits treated with adenovirus expressing human IL-1Ra had both in vitro and in vivo effects, including inhibition of IL-1 activity and inhibition of induced prostaglandin E2 synthesis. Therefore, IL-1Ra shows great promise as a gene to deliver for the treatment of arthritis.

Results from many of the above studies using IL-1Ra led the way to the first human gene therapy trials for RA which began in 1996. Using an ex vivo approach, cells removed from patients joints are transfected with retroviral vectors expressing IL-1Ra (McCarthy, 1996 and reviewed in Evan, 1998). The cells are tested for both IL-1Ra expression and for the presence of endotoxin and other agents. IL-1Ra−transduced and untransduced cells are injected back into the joints, and removed at the time of joint replacement to determine whether expression of IL-1Ra was achieved. This human trial is the first step toward assessment of local gene therapy for RA.

2. Soluble TNF receptor (sTNFR)

sTNFR is a natural inhibitor of TNF activity. Two receptors for TNF have been isolated, p55 and p75, that bind both TNFα and TNFβ (Loetscher, 1990; Smith, 1990). Soluble forms of these receptors, which are extracellular and contain ligand binding domains, inhibit TNF activity (Mohler, 1993). The sTNFR administered in clinical trials of RA is comprised of the soluble portion of the p75 cell surface receptor fused to the Fc portion of human IgG1 (sTNFRFc). The IgG1 portion prolongs the half-life of the molecule (Mohler, 1993). sTNFRFc inhibits both CIA (Mori, 1996; Williams, 1995; Wooley, 1993) and can dramatically suppress the arthitic symptoms of RA, although again, continuous administration is required (Moreland, 1997). Recently, sTNFR marketed under the trade name Enbrel (Immunex Corporation) has received approval by the FDA for the treatment of RA as a subcutaneous injection administered twice weekly.

Gene delivery of sTNFR in animal models has inhibitory effects on arthritis. In rats with CIA, systemic delivery of an adenoviral vector encoding sTNFR prior to or following the onset of arthritis, suppressed CIA. However, intra-articular administration of this vector induced an adenoviral synovitis, which was not overcome even by the expression of the sTNFR (Le, 1997). The transfer of CIA to SCID mice can also be inhibited by transducing DBA/1 spleen cells with retrovirus encoding sTNFR (Chernajovsky, 1995).

In other gene delivery studies, Ghivizanni, et al., injected adenoviruses encoding either IL-1Ra or sTNFR, both separately and in combination, into rabbit's knees (Ghivizzani, 1998). IL-1Ra reduced cartilage matrix degradation and white blood cell infiltration into the joint space. sTNFR by itself was not as effective as IL-1Ra. However, treatment with both IL-1Ra and sTNFR showed greater inhibition of white blood cell infiltration and cartilage breakdown with a considerable reduction in synovitis. In addtion, with both reagents, effects on contralateral control knees were also observed, suggesting that local intra-articular treatment may be used to treat systemic polyarticular arthritides.

B. Immune deviation

An imbalance between the activities of Th1 and Th2 cells is thought to play a role in the pathophysiology of many autoimmune diseases, such as RA. Th1 cells secrete cytokines such as IL-2 and IFN-γ, that normally mediate

pro-inflammatory immune responses, whereas Th2 cells secrete cytokines such as IL-4, lL-10, and IL-13 that can downregulate Th1 activity.

Administration of IL-4, IL-10 and IL-13 proteins to CIA mice indicate that these cytokines can inhibit the disease process (Bessis, 1996; Hesse, 1996; Horsfall, 1997; Joosten, 1997; Tanaka, 1996; Walmsley, 1996). Another animal model using streptococcal cell wall fragments to induce arthritis in rats, also demonstrates that IL-4 administration can reduce pro-inflammatory cytokine production and can inhibit experimental arthritis (Allen, 1993). In human RA synovial cells, IL-4 and IL-10 also have inhibitory effects on pro-inflammatory cytokine production (Chomarat, 1995; Isomaki, 1996; Katsikas, 1994; van Roon, 1996). These studies indicate that the Th2 type cytokines IL-4, IL-10, IL-13, which can inhibit pro-inflammatory cytokine production and the arthritic process in animal models, are good candidates for gene transfer.

1. Viral IL-10 (vIL-10) gene therapy

vIL-10 is homologous to both mouse and human IL-10 and shares many of their immunosuppressive properties, but lacks their immunostimulatory properties (Go, 1990; MacNeil, 1990). Systemic administration of adenovirus encoding vIL-10 before the onset of CIA inhibited arthritis (Apparailly, 1998; Ma, 1998), but the effects were short-term, probably due to the inflammatory response to the adenovirus. Local adminstration of vIL-10 in the footpad (Whalen, 1998) or intra-articularly into the knee (Ma, 1998) reduced the incidence of arthritis, indicating again that local gene expression can have systemic effects on disease.

2. Fas Ligand

Other methods that eliminate proliferating synovial cells are also being investigated, even though the removal of synovium has not been a successful cure for arthritis. The transduction of synovial cells with adenovirus that expresses Fas ligand induced apoptosis of synovial cells producing Fas. Administration of the virus into inflamed joints ameliorated CIA in DBA/1 mice (Zhang, 1997).

V. Future gene therapy for arthritis

Much progress has been made in recent years in the field of gene therapy for arthritis. Future efforts will be focused on determining which genes are the most promising for therapy, which vectors are the best for delivering these genes, and ultimately how to regulate expression of the genes being delivered.

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