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Gene therapy for arthritis: defining novel gene targets

Review Article

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Abbreviations: adenovirus-FasL (Ad.FasL); angiopoietin (Ang); collagen-induced arthritis (CIA); cytomegalovirus (CMV); disease-modifying anti-rheumatic drugs (DMARDs); extracellular matrix (ECM); fibroblast-like synoviocytes (FLS); helper Type 2 (Th2); inhibitor of κB (IκB); insulin-like growth factor-1 (IGF-1); Interferon-β (IFN-β); interleukin-1 (IL-1); interleukin-1 receptor antagonist (IL-1Ra); IκB kinase (IKKβ); matrix metalloproteinase (MMP); metacarpophalangeal (MCP); non-steroidal anti-inflammatory drugs (NSAIDs); osteoarthritis (OA); receptor activator of nuclear factor-κB ligand (RANKL); rheumatoid arthritis (RA); T-cell receptor (TCR); tissue inhibitor of metalloproteinases (TIMPs); TNF-α receptor-I (TNFR-I); tumor necrosis factor-α (TNF-α); vascular endothelial growth factor (VEGF); VEGF-receptor-I (VEGFR-I)

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Summary

Rheumatoid arthritis (RA) and osteoarthritis (OA) are debilitating diseases of the musculoskeletal system. RA is characterized by immune dysfunction and the classical cellular and soluble mediators of inflammation, whereas during its early stages, OA is considered a non-inflammatory disorder. However, a common pathway inherent to both RA and OA is articular cartilage and subchondral bone destruction resulting in non-functional synovial joints. Over the past decade or so, many of the candidate pathophysiologic pathways including those regulated by cytokines, growth factors and transcription factors that promote RA and OA disease progression have been elucidated. Although medical therapies directed at neutralizing cytokine activity have emerged and are now employed in treating RA and OA, there is considerable debate as to how long these biologics can be employed to modify chronic disease progression without emergent serious adverse events. This has led to a significant increase in studies that have employed gene transfer strategies in RA and OA animal models directed at inhibiting inflammatory cytokines, immune-mediated inflammation, growth factors, angiogenesis factors, apoptosis and matrix metalloproteinases that play a prominent role in RA and OA pathology. The significant abrogation of inflammation in arthritis animal models, in particular, by interleukin-1 receptor antagonist (IL-1Ra) and soluble TNF receptor gene constructs makes them potentially useful for treating RA and OA.

I. Introduction

Rheumatoid arthritis (RA) and osteoarthritis (OA) are among the most debilitating of human musculoskeletal system degenerative disorders. RA is a systemic autoimmune disease of unknown cause with cellular and humoral immune dysfunction as its hallmarks (Feldman et al, 1996; Pope and Perlman; 2000; Malemud, 2007). At the pathophysiologic level, RA is characterized principally by activation of fibroblast-like synoviocytes (FLS) within the synovial tissue which ultimately presents as synovial hyperplasia. Synovial hyperplasia results from synoviocyte proliferation, defects in apoptosis as well as lymphocyte, monocyte and macrophage migration with resultant pannus development (Malemud and Gillespie, 2005). The classical signs and symptoms of inflammation emerge from these events (Firestein, 2005), and, in RA, cartilage destruction and bone resorption result from matrix metalloproteinase (MMP) gene up-regulation and stimulation of other bone resorptive agents (Walsh et al, 2005) which result in bone destruction and ankylosis.

By contrast, the etiopathogenesis of OA is considered to be a functionally non-inflammatory process. Thus, 20 to 30 years can pass before the clinical symptoms

of OA emerge which include pain, stiffness and reduced range of motion (Veys and Verbruggen, 1999). As such, OA is a common clinical finding in the elderly population. At the cellular level, OA is characterized by mesenchymal stem cell proliferation within the synovial joint giving rise to osteophytes whose functional significance remains to be fully elucidated. However, it has been postulated that osteophytes could play a role in the reduced range of motion characteristic of OA joints (Malemud et al, 1999). Further along in the disease process, OA is typically marked by a type of inflammation involving several cellular markers of the classical inflammatory response (Pelletier et al, 2001; Attur et al, 2002a), articular cartilage remodeling, fibrillation, fissuring and dissolution (i.e. eburnation) as well as sclerotic changes in subchondral bone (Malemud et al, 2003).

An eventual pathologic feature common to both OA and RA is activation of the normal synovium (Feldmann et al, 1996). Synovial activation results in pro-inflammatory cytokine gene amplification that is, in part, regulated by stress-activated protein kinases and mitogen-activated protein kinases (Berenbaum, 2004). The resultant cytokine gene up-regulation exemplified by interleukin-1 (IL-1) and/or tumor necrosis factor-α (TNF-α) occurs without a concomitant robust stimulation of the endogenous IL-1 antagonist interleukin-1 receptor antagonist (IL-1Ra) or soluble anti-TNF-α neutralizing receptor molecule gene expression, and coupled to pro-IL-1 and pro-TNF-α activation (Attur et al, 2002a; Malemud, 2004) lead to increased levels of IL-1 and TNF-α in arthritis synovial fluid. These events ultimately result in an anabolic/catabolic imbalance with resultant joint destruction (Malemud et al, 2003). Further, stimulation of synovial angiogenic factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor-1, and -2 (Malemud 2007) as well as elevated levels of eicosanoid metabolites such as prostaglandins and leukotrienes (Martel-Pelletier et al, 1999a) accompany arthritis disease progression. In particular, IL-1 and TNF-α both stimulate chondrocyte matrix metalloproteinases (MMPs) as well as a family of proteolytic enzymes with the structural properties of a disintegrin and metalloproteinase with thrombospondin motif (ADAMTS) gene expression while suppressing endogenous MMP inhibitors such as the family of tissue inhibitor of metalloproteinases (TIMPs) (Burrage et al, 2006). The concerted action of several MMPs, most prominently, MMP-2, MMP-3, MMP-9 and MMP-13 (Burrage et al, 2006) in combination with reduced or only slightly elevated TIMP levels appear to be responsible for cartilage extracellular matrix (ECM) protein degradation in arthritis (Burrage et al, 2006).

Adding to cartilage and bone deterioration, a common finding in RA and OA, is ECM protein synthesis suppression. ECM protein suppression occurs as a result of pro-inflammatory cytokine activity as well as the activity of other soluble mediators of inflammation which activate transcription factors causing a decrease in ECM protein gene expression (Martel-Pelletier et al, 1999b; Fernandes et al, 2002). In addition, chondrocyte non-responsiveness to circulating growth factors such as insulin-like growth factor-1 (IGF-1) also occur which likely compromise articular cartilage and subchondral bone repair pathways. The possibility that a skewed upward growth hormone to somatostatin ratio plays a critical role in RA inflammation (Denko and Malemud, 2004) and that pituitary/hypothalamic dysfunction in general regulates, in part, arthritis inflammation, pain and disease progression has also recently been reviewed (Denko and Malemud, 2005).

Medical therapies designed to retard arthritis progression have commonly included anti-inflammation strategies such as oral and intra-articular corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs) (Pelletier et al, 1994) as well as disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate, hydroxychloroquine, sulfasalazine and leflunomide (Gaffo et al, 2006). More recently, anti-IL-1 [i.e. IL-1 receptor antagonist; anakinra; (Kineret)®], anti-TNF-α neutralizing monoclonal antibodies (i.e. infliximab; (Remicade®); adalimumab; (Humira®), **dimeric p75TFNR fusion protein** (i.e. etanercept; Enbrel®), anti-T-cell activation (i.e. abatacept; Orencia®) as well as anti-CD20 therapy (i.e. rituximab; Rituxan®) have been employed primarily in RA (Gaffo, 2006). However, a common problem likely to occur in treating OA and RA patients with these biologic agents is the deleterious side-effects and additional co-morbid conditions, in particular, infections such as tuberculosis (Weaver, 2003) as well as other emergent medical problems such as malignancies and thrombocytopenia that may associate with employing these biologic agents over the long duration of treating these chronic diseases especially with anti-TNF-α biologics.

Testing of experimental agents in RA and OA animal models that are designed to suppress cartilage ECM degradation and bone resorption has generally involved oral administration of synthetic MMP inhibitors and other anti-resorption compounds with less than ideal efficacy or amelioration of clinicopathologic features (Malemud et al, 2003; Malemud, 2004). Because of the generally unfavorable results obtained by attempting to suppress MMP activity in arthritis animal models, alternative modalities including gene therapy strategies have been developed that stem from elucidating the key intracellular pathways giving rise to inflammation, apoptosis resistance, ECM protein degradation and suppression of ECM protein compensatory biosynthesis in arthritis (Malemud et al, 2003). In addition, successful experimental therapy of arthritis in animal models with recombinant proteins have generally required continuous, multiple injections to achieve any amelioration of clinical inflammation (van de Loo et al, 2006; Moritz et al, 2006; Adriaansen et al, 2006a). Several of these approaches have involved local administration of recombinant proteins to affected joints. However, the identification of novel targets for RA and OA intervention in animal models notwithstanding (Moritz et al, 2006; van de Loo et al, 2006) the problem of achieving sustained biological effects by comparing local with systemic administration of recombinant proteins has generally favored the former rather than the latter mode of recombinant protein

administration. Additional overriding issues of potential clinical toxicity and lack of efficacy inherent in the use recombinant proteins have led to a more sustained and systematic effort to employ gene therapy for arthritis intervention.

This review will focus on significant developments in gene therapy strategies for arthritis since 2003 in which gene transfer has been employed to suppress arthritis incidence, progression and pathophysiologic pathways in arthritis animal models. The generally favorable results of gene transfer studies in animals (Campbell et al, 2005; van de Loo et al, 2006) have led to their consideration for use in phase I clinical trials in OA (Evans, 2005; Evans et al, 2006) and RA (Tomita et al, 2003; Muller-Ladner et al, 2003; Van de Loo, 2004, Van de Loo et al, 2004; Adriaansen et al, 2006a; Nakajima, 2006).

From an historical perspective on the use of gene transfer to treat experimental OA, earlier studies where *in vivo* gene transfer of an IL-Ra gene-construct (Pelletier et al, 1997; Fernandes et al, 1999) or an IL-1β-receptor decoy gene (Attur et al, 2002b) was employed should be appreciated. These gene constructs were successfully implemented in OA animal models and additional *in vitro* studies also suggested a mechanism for their action.

II. Anti-cytokine approaches

A. IL-1Ra gene transfer

Ever since cytokines were identified as playing a key role in OA and RA disease progression, these molecules have been targeted for gene therapy intervention in arthritis animal models with a view towards applying the results of these studies to phase I clinical arthritis trials. In that regard, IL-1Ra cDNA was shown to reduce inflammation in an RA animal model characterized by a deficiency in IL-1Ra (Hur et al, 2006). In particular, an adenoviral vector-construct containing human hIL-1Ra and GFP (i.e.Ad.IL-1Ra.GFP) was administered by intra-articular injection into the ankle joints of mice with established chronic inflammatory arthritis induced by Type II collagen (i.e. collagen-induced arthritis; CIA). Not only did the treated mice show abrogation of arthritis inflammation, but these animals also showed reduced levels of helper Type 1 (Th1)-driven IgG2a antibodies to Type II collagen compared to controls and increased levels of helper Type 2 (Th2)-driven IgG1 antibody as well. These studies established that gene transfer of IL-1Ra could be therapeutically efficacious in suppressing the immunologically-mediated early stages of human RA.

In a more recent short-term phase I clinical trial, 9 postmenopausal women with destructive RA underwent unilateral silastic implant surgery. Synovium was recovered from the 2^{nd}-5^{th} metacarpophalangeal (MCP) joints and RA FLS transduced with a retrovirus containing IL-Ra cDNA *ex vivo*. After intra-articular injection of autologous FLS-containing the IL-1Ra transgene (10^6-6.5-10^6 cells) into 2^{nd}-5^{th} MCP operated joint of one hand, the IL-1Ra transgene was expressed as IL-1Ra protein at levels significantly higher than joint tissues injected with control vector, but IL-1Ra expression occurred only at the highest cell dose (Evans et al, 2005). Although no adverse events occurred, intra-articular injection did produce unwarranted side-effects in some of the study participants characterized as injection site reaction with echymosis. In addition, because leukemia had developed in some children with SCID using cell therapy (Check, 2003) and the same viral backbone employed in the study reported by Evans and colleagues in 2005, a 5-year waiting period ensued before publication of the study results to ensure that unwarranted effects of the IL-1Ra gene transfer did not occur. After more than 5 years, the subjects receiving the IL-1Ra transgene were free of replication-competent virus and no adverse events occurred. These results showed the feasibility of direct gene transfer of FLS containing an IL-1Ra transgene pertinent to suppressing cartilage and bone destruction in human RA.

B. IL-10 gene transfer

IL-10 is a cytokine with potent anti-inflammatory properties and the capacity to down-regulate IL-1 and TNF-α *in vitro* (Fernandes et al, 2002). Of note, IL-10 also suppressed TNF receptor-I expression while increasing the level of TNF receptor-II in human FLS isolated from OA synovium (Alaaeddine et al, 1999). Thus, the capacity to increase IL-10 levels by gene transfer seemed to be a worthy target in RA and OA. In that regard, Woods and colleagues showed in 2005 that when used prophylactically, intranasal delivery of an IL-10 gene-construct (pG-IL-10) to DBA/1 mice with CIA delayed arthritis onset and reduced arthritis severity. IL-10 expression appeared to target monocytes and macrophages as well as draining lymph nodes. The expression of pG-IL-10, however, appeared to be independent of any changes in TNF-α. In addition, Khoury and colleagues showed in 2006 that delivery of an IL-10 gene construct using a non-viral *in vivo* intra-muscular electrotransfer method to mice with CIA was more effective in suppressing inflammation than intra-articular electrotransfer administration.

C. IL-13 gene transfer

IL-13 is a Th2-produced cytokine with potent anti-inflammatory properties that is abundant in the synovial fluid of RA patients (Malemud, 2004). *In vitro*, IL-13 inhibited IL-1β and TNF-α expression and augmented IL-1Ra production in human OA-FLS stimulated with lipopolysaccharide (Jovanovic et al, 1998). In a recent study, Nabbe and colleagues showed in 2005 that an IL-13 gene construct transferred using an adenoviral vector to mice with immune complex-induced arthritis actually increased the number of synovial joint inflammatory cells. However, IL-13 gene transfer also abrogated chondrocyte apoptosis via down-regulation of FCγRI and MMP-mediated proteoglycan degradation induced by immune-complexes without altering MMP-3, -9, -12 or -13 mRNA levels. These results suggested that IL-13 over-expression in immune-complex-mediated murine arthritis inhibited chondrocyte apoptosis and proteoglycan cleavage despite having little effect on the overall inflammation profile. These studies concluded that modulation of FCγRI by TH2 cytokines might provide an additional therapeutic approach for modifying cartilage damage in experimental RA (Nabbe et al, 2005).

D. IL-4 gene transfer

IL-4 is another anti-inflammatory cytokine that also can spare synoviocytes from nitric oxide-induced apoptosis *in vitro* (Relic et al, 2001). IL-4 has also been shown to suppress TNF-α-mediated prostaglandin E_2 production by OA synovial fibroblasts (Alaaeddine et al, 1999). In a recent study, Haas and colleagues showed in 2006 that an IL-4 gene transferred to rats with adjuvant-induced arthritis significantly reduced synovial vessel density and ankle joint inflammation. Joint homogenates collected from rats containing the IL-4 transgene inhibited both endothelial migration and tube formation *in vitro* despite high levels of VEGF. The angiostatic effects of the IL-4 transgene correlated *in vitro* with elevated levels of IL-18, chemokines CXL16 and CXL5 as well as endostatin. Thus, IL-4 transgene strategies that demonstrate suppression of synovial neoangiogenesis might provide some efficacy in RA.

E. Neutralizing TNF-α by gene transfer

Anti-TNF-α monoclonal antibody therapy has proven efficacy as an RA therapy (Hsu et al, 2006). Anti-TNF-α-mediated suppression of inflammation is regarded as the major mechanism attributed to these therapies which have also been shown to retard radiologic evidence of RA disease progression, especially when employed in conjunction with methotrexate (Breedveld et al, 2006). However, there is little evidence that any of the current RA therapies with DMARDs or biologics reverse the damage to joints caused by RA, although aggressive combination therapy (e.g. adalimumab plus methotrexate) appeared to be superior to monotherapy (e.g. adalimumab or methotrexate) in retarding RA progression (Breedveld et al, 2006).

At the cellular level, the mechanisms attributed to anti-TNF-α strategies primarily include, a reduction in circulating TNF-α, suppression of nuclear factor-κB activation and down-regulation of MMP gene transcription (Berenbaum, 2004; Roman-Blas and Jimenez, 2006), making use of anti-TNF-α treatment potentially attractive for the therapy of OA as well as RA. Because it is not known how long RA patients can be treated with the current commercially available anti-TNF-α biologics, infliximab, etanercept and adalimumab, that neutralize the downstream effector pathways attributed to TNF-α makes TNF-α gene therapy strategies an attractive target for arthritis intervention. In that regard, Bloquel and colleagues showed in 2004 that a single dose of plasmids encoding 3 soluble TNF-α receptor-I (TNFR-I) variant forms (i.e. monomeric, dimeric and chimeras) delivered intra-muscularly by electrotransfer to mice with CIA reduced the clinical signs of arthritis inflammation. However, no change in arthritis clinical scores occurred when only the monomeric form of the soluble TNFR-I transgene was employed and only moderate changes occurred with the dimeric form. Thus, it appeared that at least 2 of the 3 variant subunits of TNF-α trimer have to bind to inhibit cell activation by TNF-α. Prevention of transmembrane TNF receptor binding was proposed as the putative mechanism of action of these TNFR-I-receptor constructs because the remaining TNF subunit was unable to activate transmembrane TNF-RI in keeping with a model previously proposed by Adam and colleagues, in 1995. In addition, the anti-inflammatory effect of the TNFR-I receptor-construct was long-lasting and compared favorably with repeated intra-muscular treatments with etanercept. These results suggested that targeting TNF-α receptor-I by gene transfer may also prove useful in future arthritis therapy. In addition to studies focusing on the use of soluble TNF-RI gene therapy strategies, there are additional ongoing clinical trials with soluble TNF-RII (see, http://www.wiley.co.uk/genetherapy/clinical).

III. Immunomodulatory approaches

A. Interferon-β

Interferon-β (IFN-β) has potent immunomodulatory properties (Tak, 2004). Its use as a human RA therapy was, however, compromised in two recent clinical trials by apparent attrition problems due to lack of efficacy rather than safety (Genovese et al, 2004) and the failure of subcutaneous administration of IFN-β1 together with methotrexate to alter RA radiographic progression (van Holten et al, 2005). This lack of efficacy may also be attributable to the mode of administration as well as issues of pharmacokinetics (Adriaansen et al, 2006b), even though a prior study had shown that daily recombinant IFN-β injections reduced arthritis severity and inhibited pro-inflammatory cytokine responses, including TNF-α and IL-6, while increasing IL-10 in murine CIA (van Holten et al, 2004). To investigate the possibility that IFN-β gene transfer could substitute for administration of recombinant INF-β protein, Adriaansen and colleagues, (2006b) treated rat AIA with an intra-articular injection of adenovirus vector containing an IFN-β construct (i.e. Ad.IFN-β) at different doses ranging from 1.2×10^9 to 1.2×10^{11} viral particles. The levels of IFN-β synthesis in the arthritic hindpaw peaked 2 days after intra-articular Ad.IFN-ß injection and synovial inflammation was significantly reduced. Further, Ad.IFN-β abrogated bone erosions, but only at the highest dose employed (i.e. 1.2×10^{11} viral particles). In addition, Ad.IFN-β reduced the signaling molecules, c-Cbl and CBl-b whose activity is critical for osteoclast differentiation (Miyazaki et al, 2004) as well as reducing the MMP-3/TIMP-1 ratio. A histologic examination of the rat synovium suggested an anti-inflammatory effect of Ad.IFN-β.

B. T-cell Receptor (TCR) strategies

T-cells containing both TCRα and TCRβ accumulate in human RA synovium (Firestein, 2005) whereas the TCRβ gene repertoire provided a signature profile for T-cell involvement in the inflamed paws of mice with CIA (Haqqi et al, 1992). To investigate the extent to which TCR recovered from the inflamed joints of mice with CIA could then be employed to suppress inflammation, Fujio and colleagues in 2006 first expanded a clone of cells containing TCRα,β genes that were originally isolated from the inflamed paw of a mouse with CIA. The clonotype (i.e. B47) was reconstituted on peripheral $CD4^+$ T-cells and used as the therapeutic cellular vehicle. B47 was autoreactive, but not specific for Type II collagen.

Fujio et al, (2006) found that TCR genes from B47-transduced T cells accumulated in the inflamed paws of mice with CIA. Of note, injection of cells that had been co-transduced with B47 and a soluble TCRRIg gene-expressing vector by fusing the p75 TNFR (i.e. TNFR-II) with the F_c domain of $IgG2_a$ also resulted in significant suppression of paw inflammation. Arthritis suppression correlated with TNFRIg transcripts in the hind paw but not with serum TNFRIg content. In addition, cells co-transduced with B47 and a Foxp3 gene-construct reduced levels of TNF-α, IL-17A, IL-1β, suppressed bone destruction and progression of established CIA. These studies suggested that TCR genes might be applied to induce immune suppression in inflammatory arthritis.

IV. Anti-angiogenesis strategies

A. Tie-2

RA progression is dependent on neoangiogenesis (Malemud, 2007). The angiopoietin (Ang)-Tie ligand receptor system plays a key role in vascular integrity by virtue of its ability to regulate permeability, resistance, and, in arthritis to regulate inflammation by its involvement in synovial neoangiogenesis (Fiedler and Augustin, 2006). Tie-2 and Ang-1 were previously shown to regulate angiogenesis in CIA by virtue of the fact that Tie-2 and Ang-1 levels were increased in RA synovium (DeBusk et al, 2003). Further, DeBusk and colleagues found in 2003 that TNF-α promoted Tie-2 deposition in synovium which apparently involved interactions between endothelial cells and FLS, NF-κB activation and Ang-1 expression. The results of that study (DeBusk et al, 2003) provided the impetus for employing an adenovirus construct containing a soluble Tie-2 receptor (i.e. Ad.ExTek) to treat murine CIA. Thus, Chen and colleagues showed in 2005 that CIA disease severity was considerably reduced when Ad.ExTek was administered after the onset of CIA compared to the control group. Further, Ad.ExTek protected mice with CIA against bony erosions, suppressed neoangiogenesis in the paws of Ad.ExTek-treated mice and reduced the levels of receptor activator of nuclear factor-κB ligand (RANKL), a key mediator of osteoclastogenesis and bone erosion. However, no differences in anti-collagen antibodies were noted and suppression of CIA disease activity could not be correlated with suppression of anti-collagen antibodies.

B. VEGF

VEGF-B is a significant mediator of neoangiogenesis in RA synovium (Malemud, 2007). In CIA, the arthritis incidence and severity was greatly diminished in VEGF-B -/- mice compared to their wild-type littermates as was synovial vessel density (Roccaro et al, 2005). Further, the finding that an adenoviral VEGF-receptor-I (VEGFR-I)-construct delivered to mice with CIA significantly diminished arthritis severity suggested that VEGF blockade via VEGFR-I gene transfer (Afuwape et al, 2003) could be a successful angiostatin mechanism in experimental arthritis.

C. Kallistatin

Kallistatin is a member of the serpin protein family that was identified as a specific inhibitor of kallikrein (Miao et al, 2003). Whereas kallikrein was shown to promote angiogenesis (Emanueli et al, 2002), kallistatin was shown to inhibit angiogenesis and tumor growth (Miao et al, 2002). This evidence suggested that kallistatin might be a potent inhibitor of VEGF-mediated angiogenesis in RA. Thus, Wang and colleagues, showed in 2005 that an adenoviral vector containing the human kallistatin gene (i.e. Ad.H.KBP) suppressed rat ankle arthritis, reduced synovial vessel density and neutrophil levels as well as IL-1β and TNF-α, but not VEGF. In addition, Ad.H.KBP markedly inhibited endothelial proliferation *in vitro* suggesting that kallistatin gene transfer could become an adjunctive angiostatin therapy in RA.

V. Stimulation of apoptosis by gene transfer

Defective synovial apoptosis with resultant synovial hypertrophy is one of the hallmarks of RA, and may arise as a result of defects in Fas/Fas ligand-induced apoptosis (Perlman et al, 2001; Baier et al, 2003; Malemud and Gillespie, 2005). Previously, FasL mRNA could be detected in 5 of 6 RA patient synovium but most of the FasL was localized to infiltrating mononuclear cells (Asahara et al, 1997). Thus, correcting a putative defect in the Fas/FasL pathway by FasL gene transfer might be an effective "molecular" substitute for surgical synovectomy in RA. Recently, Kim and colleagues showed in 2006 that synovectomy of hand RA moderately improved hand function although no changes in grip strength or range of motion occurred. Further, synovectomy was not recommended for the RA knee or ankle with radiographically advanced joint space narrowing (Kim et al, 2006). To address the possibility that FasL gene therapy could ameliorate synovial hypertrophy, Zhang and colleagues studied in 2005 whether FasL gene transfer could alter the inflammatory properties of human RA synovia obtained by synovectomy. Zhang and colleagues in 2005 employed repeated administrations of adenovirus-FasL (i.e. Ad.FasL) *ex vivo* and then grafted Ad.FasL-transduced synovia on to C.B-17 SCID mice. After recovering the implants, the number of synoviocytes and mononuclear cells was significantly reduced compared to implants from control animals and an approximately 15-fold increase in apoptotic nuclei was seen. This study suggested that intra-articular FasL gene transfer could serve to induce apoptosis in RA synovium and could therefore be conceivably employed to reduce the effects of synovial pannus in RA.

VI. NF-κB gene therapy

Suppression of NF-κB activation is a critical step in modulating the inflammatory response (Malemud et al, 2003). Phosphorylation of the inhibitor of κB (IκB) is a prerequisite event in NF-κB activation during inflammation which is principally regulated by IκB kinase (IKKβ) (Chen et al, 2001; Hayden and Ghosh, 2004).

Thus, Tas and colleagues (2006) specifically targeted IKKβ by employing a recombinant adeno-associated virus type 5 dominant-negative-IKKβ-construct (i.e. rAAV5.IKKβ.dn) by intra-articular injection into the inflamed right ankle joint of rats with AIA. The results showed that right ankle paw swelling was suppressed and immunohistochemical analysis of the synovium revealed reduced intensity of IL-6 and TNF-α, but not IL-10. However, no significant effect was found on cartilage and bone destruction, MMP-3 or TIMP-1. This study showed that rAAV5.IKKβ could be successfully employed to locally reduce experimental arthritis inflammation endpoints *in vivo* and *ex vivo*. Although rAAV generally transduces less efficiently than adenoviral vectors expressing the same transgene, stable long-term expression is posited to make rAAV a better candidate than adenovirus for gene transfers in chronic diseases such as RA (Chernajovsky et al, 2004).

VII. TIMP gene therapy

TIMPs are the endogenous MMP inhibitors that maintain normal synovial joint homeostasis and are critical in modulating MMP activity during skeletal development (Malemud, 2006). In early RA, serum MMP to TIMP ratios are generally skewed upward, especially MMP-3/TIMP-2 and MMP-9/TIMP-2, compared to an OA control group (Fiedorczyk et al, 2006). This result is likely to be pertinent to the aggressive MMP-mediated degradation of RA cartilage and bone ECM proteins. Of note, methotrexate treatment in RA patients did not alter the MMP-3, -9/TIMP-2 ratios (Fiedorczyk et al, 2006). In OA, synovial fluid MMP levels are also increased (Malemud, 1999) but the mRNA of some TIMP family members (i.e. TIMP-3) are also increased whereas TIMP-1 and TIMP-4 mRNA are decreased (Kevorkian et al, 2004). To address the possibility that restoration of TIMP levels could ablate MMP effects in inflammatory arthritis, van der Laan and colleagues in 2003 used adenoviral-TIMP-1 and TIMP-3 gene transfer to show that TIMP-1/TIMP-3 over-expression resulted in significant suppression of proliferation and invasiveness in RA-FLS *in vitro* and also after engrafting on to SCID mice *in vivo*. Although these studies using SCID were limited with respect to their long-term application to human RA, they may provide the impetus for additional studies in which a TIMP gene transfer to pannus might support a mechanism for suppressing MMP activity and cartilage and bone destruction of RA at that site.

VIII. IGF-1

Chondrocyte unresponsiveness to IGF-1 is considered significant in OA because IGF-1 is crucial for cartilage and bone repair pathways (Denko and Malemud, 2005). To address the possibility that IGF-1 gene therapy could subserve IL-1Ra in OA, Nixon and colleagues in 2005 co-transduced synovial membrane with E1-deleted adenoviral vectors, one containing IGF-1 sequences under cytomegalovirus (CMV) promoter control and the other with IL-1Ra under CMV promoter control. Transduced IGF-1/IL-Ra synovium showed increased IGF-1 and IL-Ra mRNA. Further, the IGF-1 protein concentration collected from synovial cell monolayer cultures was sufficient to stimulate proteoglycan synthesis by normal cartilage explants. This study suggested that IGF-1/IL-Ra co-transduction of synovial membrane may be a feasible gene therapy model for stimulating cartilage ECM proteins during early arthritis.

IX. Conclusions

Although several medical therapies appear to be provide symptomatic relief of pain in RA and OA, at present, specific therapies that take advantage of the pathophysiologic events pertinent to RA and OA focus on neutralizing the effects of IL-1 (i.e. IL-1Ra) and TNF-α with soluble TNF receptor monoclonal antibodies. An approach using dominant negative TNF-variants has also been considered (Steed et al, 2003). More recently, targeting the B-cell antigen, CD20 with rituximab has been tested in RA (Dass et al, 2006; Popa et al, 2006). Since 2003, experimental studies employing a variety of gene therapy strategies have shown that over-expression of transferred genes that inhibit cytokines, growth factors and MMP activity as well as immune-mediated events and inflammation may be useful in the future treatment of RA and OA. However, additional animal studies are warranted so that the long-term safety as well as temporal and spatial consequences of continuous expression of transferred gene-constructs can be monitored. Successful exploitation of IL-1Ra and IGF-1 gene transfers in human tissue *in vitro* (i.e. the *ex vivo* 'approach') has resulted in the generation of IL-1Ra and IGF-1 gene transfer strategies for eventual use in phase I OA clinical trials (Tomita et al, 2003; Evans, 2005).

The success or failure of specific gene therapy strategies for RA and even OA is likely to depend on the type of vectors employed (Bessis and Boissier, 2006) as well as host immune responses to the transgene including stimulation of innate immunity (Bessis et al, 2004). It also remains to be determined if, for example, rheumatoid factors with the capacity to form immune complexes with the expressed secreted protein directed by the transgene will limit their effectiveness in ameliorating immune cell dysfunction and inflammation in RA and cartilage destruction in OA (Evans et al, 2005).

Gene therapy for arthritis may be the 'wave of the future' and clinical trials are warranted to test the efficacy of gene transfer paradigms determined from animal studies. These would include the effectiveness of adeno-associated viral vectors compared to adenoviral vectors and local administration compared to systemic injection. However, it is still too early to predict the extent to which gene therapy will become an adjunctive arthritis treatment or even replace the current standard of medical care employed in the treatment of clinically active RA or OA (Gaffo et al, 2006).

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