Gene therapy for rheumatoid arthritis

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Therapy for rheumatoid arthritis (RA) has seen significant advances over the past decade. The use of biologic agents, such as TNF-α inhibitors, have led to improvement in up to 60% of RA patients. Unfortunately, biologic therapy also presents significant limitations, including systemic side effects, a short half-life with requirement for frequent dosing, and a lack of curative response. Owing to such limitations, the ideal therapy for RA remains unrealized. Progress in the field of gene therapy provides interesting and applicable methods to overcome many of these deficits. In this review we will discuss some of these advances, focusing on the development of new vectors, gene-therapy targets and regulatory mechanisms. With continued efforts in this field, the hope for a lasting, regulated and possibly curative modality appears attainable.

Arthritis is the leading cause of disability in the USA, affecting one out of five (46 million) adults [201]. Rheumatoid arthritis (RA), a chronic, destructive inflammatory arthritis, is a major subtype with a worldwide prevalence ranging from 0.2% to greater than 1% in North America [1]. Advances in understanding the pathophysiology of RA have led to the development of novel therapeutic biologic agents, such as TNF-α and IL-1 inhibitors. Importantly, these treatments have demonstrated efficacy in 60% of RA patients [2]. Despite the many benefits of these systemic agents, their side effects, short duration of effect, need for long-term treatment and inability to cure the disease, cause them to fall short of optimal RA therapy. For these reasons, the development of targeted gene therapy is an increasingly attractive option for long-term RA disease control. In order to make gene therapy a viable therapeutic option for RA the following need to be optimized: gene-delivery, vectors, candidate molecules and targets and methods to regulate transgene expression.

Gene delivery approaches to arthritis

Gene delivery for the treatment of RA can be performed either locally or systemically. Local injection into the joint is a minimally invasive method to deliver high amounts of therapeutic drug directly to areas of inflammation. Although vector spread, transgene protein leakage and trafficking of transduced fibroblast-like synovial cells, leucocytes or antigen-presenting cells (APCs) may produce therapeutic responses at distant sites, the extent and efficacy of this distal effect following local injection is highly variable. In this light, systemic application is attractive in its potential to target multiple sites of disease activity with a single application. For RA this is particularly desirable owing to the propensity of RA to present with multiple joint involvement. Of concern, however, is that increased vector doses required for systemic therapy may heighten the risk for vector and transgene protein side effects. As a result, an optimal scenario is one that combines the ability to deliver moderate doses locally with targeted spread to other areas of inflammation, or systemic delivery with selective homing to areas of disease activity.

Three delivery approaches for RA gene therapy have been proposed and tested experimentally (**Figure 1**). The first, *in vivo* gene delivery, involves direct injection of transgene vectors, usually viral, into the host's target tissue. *In vivo* delivery minimizes manipulation of the target cell population, typically synoviocytes, by directly targeting these cells in their normal host environment. Despite the attractive nature of *in vivo* therapy, two major disadvantages exist: limited transduction efficiency and transient transgene expression. Limited transduction efficiency largely results from the induction of a host immune response against the vector and infected cells. Transient expression results from the inability of most vectors to integrate permanently into the host genome, leading to a loss of expression with successive cell divisions.

The second delivery approach, *ex vivo* gene delivery, involves the harvesting and expansion of target cells, followed by *in vitro* transduction and reimplantation back into the host. The major advantage over *in vivo* systems is the ability to select and expand stably transduced cells prior to reinjection. Such controlled gene insertion

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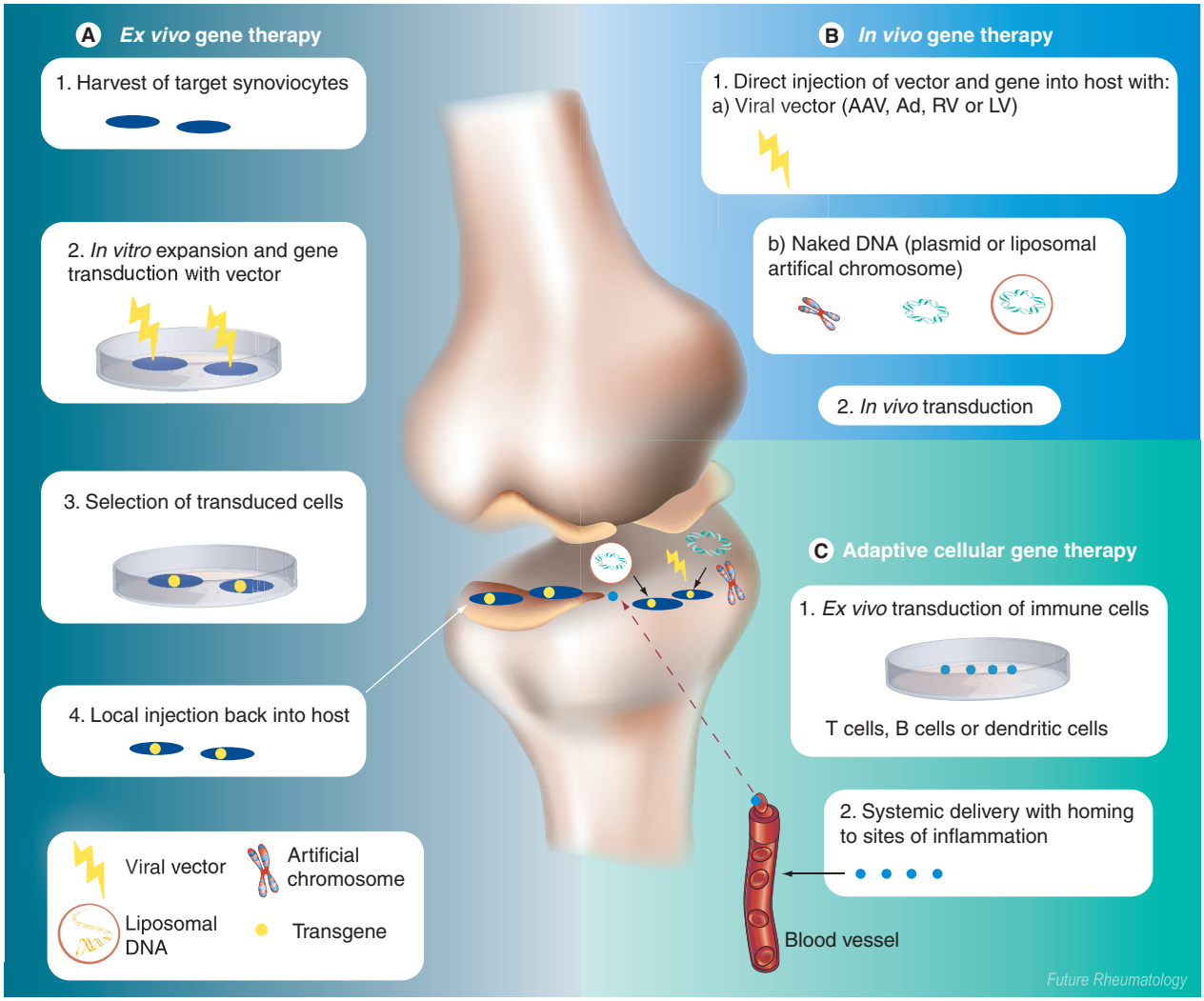
Figure 1. The three most common approaches to gene therapy in rheumatoid arthritis.



A) *Ex vivo* gene therapy shows *in vitro* transduction of synoviocytes followed by implantation back into host. **B)** *In vivo* gene delivery represented by direct transfer of transgene-expressing vector into the host followed by *in vivo* transduction. **C)** Adoptive cellular gene therapy shown as systemic intravenous administration of immune cells carrying the target therapeutic gene with homing to joint inflammation. AAV: Adeno-associated virus; Ad: Adenoviral: LV: Lentivirus; RV: Retrovirus.

and selection can then lead to longer lasting transgene expression. Furthermore, host immune activation is more easily evaded through the avoidance of exposure to viral vectors. Disadvantages include increased costs, low levels of survival of implanted cells and the need for additional invasive procedures required to harvest synovium and implant target synoviocytes.

Finally, adoptive cellular gene therapy (ACGT) provides an alternative *ex vivo* approach using immune cells, such as T cells, B cells or APCs, as vectors for gene delivery [3–5]. The promise behind ACGT lies in the ability of these immune cells to migrate to distant sites of inflammation following systemic or local administration [5]. Currently engineered T cells designed to target joint-specific antigens have proven effective in reducing inflammation in mouse models of arthritis [6,7].

Gene delivery vectors for arthritis

Viral vectors

Retrovirus

Viral vectors currently provide the most efficient system for high-level transgene expression *in vivo*. Retrovirus (RV) and lentivirus (LV) vectors are commonly used vehicles for *ex vivo* gene transfer owing to their ability to integrate transgenes directly into the host genome, providing the advantage of stable expression. Several

studies have shown effective transduction and protein expression within human synoviocytes using RV/LV vectors [8–11]. Drawbacks to the use of RV vectors include the poor transduction of nondividing cells and poor *in vivo* transduction, although there has been some success with direct *in vivo* transfer at very high retroviral titers [12,13]. Lentiviral vectors, derived from RVs, have the benefit of infecting quiescent cells by penetration through the nuclear membrane. Another concern with RV/LV vectors is the risk for mutagenesis as a result of random genome insertion [14].

Adenovirus

First-generation recombinant adenoviral (Ad) vectors, lacking viral replication genes, are able to transduce dividing and nondividing cells and can be produced in high titer, providing an attractive option for both *in vivo* and *in vitro* systems. A major limitation for the use of Ad vectors in gene therapy stems from their induction of a significant host immune response after infection. In addition, up to 70% of RA patients acquire neutralizing antibodies in their synovial fluid [15]. The inability of Ad vectors to permanently integrate into the host genome, leading to loss of transgene expression with each cell division, further limits their utility for long-term expression. Advances in recombinant Ad vectors, including engineered vectors that lack immunogenic viral capsid proteins and vectors with modified capsids that target synoviocyte surface receptors [16,17], may improve the utility of Ad as a gene delivery vehicle for arthritis.

Adeno-associated virus

Adeno-associated virus (AAV) is less immunogenic than Ad and lacks immunogenicity in humans. Over 100 AAV isolates have been identified, with 11 being best characterized. Eight serotypes, AAV1–5 and AAV7–9, are regarded as true serotypes in their inability to cross-react with neutralizing sera specific for all other characterized serotypes [18]. Of the true serotypes, AAV2 has been most extensively studied in gene therapy, leading to its approval in several clinical trials in cystic fibrosis, hemophilia and, recently, RA [19]. Despite its efficacy and low side-effect profile, a major limitation has been the finding that up to 30–80% of the normal adult populations' and 50–70% of RA patients' synovial fluid may have neutralizing antibodies against AAV2 [20–22]. Furthermore, anti-AAV2 IgG isolated from RA synovial fluid effectively neutralized *in vitro* infection of chondrocytes [22]. Although some studies have demonstrated similar percentages of neutralizing antibodies to AAV5 [15], a recent direct comparison of synovial fluid neutralizing antibodies to AAV-5 and -2 showed a lower incidence of neutralizing antibodies (10 vs 50%) [20]. This lower immunity may explain other studies demonstrating a higher synovial transduction efficiency in comparison with AAV2 [23], making AAV5 a promising alternative to AAV2. Interestingly, intra-articular injection of recombinant (r)AAV-2/5, a pseudotype containing AAV2 terminal repeats flanking the reporter gene within an AAV5 capsid, led to earlier and longer expression than AAV-2/2 [24]. Such pseudotype engineering may provide an improved therapeutic option for patients with neutralizing antibodies.

Nonviral vectors

Multiple nonviral vectors have been developed with the primary benefit of having decreased immunogenicity and host toxicity compared with their viral counterparts. Plasmid DNA can be produced on a large scale via bacterial sources and is able to deliver large or multiple gene sequences to the target cell. Unfortunately, the use of naked DNA is susceptible to rapid degradation within target cells, limiting transduction efficiency and expression in comparison with viral vectors [25]. Despite this, the electrotransfer of naked human IL-1 receptor antagonist (IL-1Ra), TNF-α receptor and IL-10 plasmid DNA into skeletal muscles, and abdominal gene-gun delivery of plasmid IL-4 have been effective in animal models of arthritis [26–30]. Recently, electric-pulse delivery following local joint administration of a human IL-10 plasmid and siRNA to TNF-α in mouse arthritis models have shown further potential for their use in the treatment of arthritis [31,32].

The artificial chromosome expression (ACE) system has potential advantages over naked DNA in its ability to maintain DNA episomally for longer periods and theoretically minimizing the risk of random host genome integration. *Ex vivo* delivery of ACE into rat synoviocytes led to long-term reporter gene expression following injection into knee joints in an adjuvant arthritis mode. The major limitation of ACE in this study was poor transfection rates, with uptake in only a quarter of cultured cells, and the lack of stable gene

expression in rat synoviocytes compared with skin fibroblasts. It is likely that the poor transfection resulted from ACE's lack of necessary machinery to enter cells on their own, while the lack of stable gene expression in synoviocytes resulted from increased toxicity to the ACE system and slower growth compared with other primary lines [33].

Candidate genes

Inhibition of proinflammatory cytokines

Inhibition of TNF-α using the soluble TNF receptor entanercept, or an antibody to TNF (infliximab or adalimumab), has shown efficacy in multiple RA trials [2]. However, frequent treatment is required to sustain response. Delivery of soluble TNF receptor, both by AAV vectors and by electrotransfer of plasmid DNA, has shown efficacy in decreasing joint erosions and synovial hypertrophy in mouse models [26,34,35]. Early human data from a Phase I/II clinical trial showed a trend for decreased swelling and tenderness in 40 RA patients treated with intra-articular injection of tgAAC94, an rAAV2 vector encoding a human TNF receptor (TNFR)-immunoglobulin (IgG1) Fc fusion (*TNFR:Fc*) gene [36].

Similar to TNF, increased expression of IL-1 or diminished expression of IL-1Ra in mice led to a highly aggressive form of arthritis similar to chronic RA in humans [37,38]. Although the use of anakinra, a recombinant IL-1Ra, has been shown in multiple clinical trials to be effective in moderating RA, its short half-life and requirement for daily injections make longer-lasting gene therapy attractive. *Ex vivo* modification of synoviovytes with retrovirus encoding IL-1Ra is both chondroprotective and anti-inflammatory in rodent models. A clinical study in which retrovirally transduced autologous synoviocytes were injected into metacarpophalangeal joints of patients with RA prior to joint replacement showed consistent expression of IL-1Ra [39,40]. *In vivo* delivery of IL-1Ra into the knee joints of arthritic rats using AAV vectors led to decreased joint inflammation [41]. Lastly, just as IL-1 inhibition with anakinra has shown additive effects in patients with an incomplete response to TNF inhibition or methotrexate alone, coadministration of soluble TNFR and IL-1 antagonist was more efficacious than on IL-1 antagonist alone [35].

Recent findings showing that proinflammatory cytokines IL-17 and -18 act independently of IL-1 to contribute to inflammatory arthritis, have provided an additional target for RA therapy [42,43]. Both Ad-vector delivery of the IL-18 binding-protein gene (*AdCMVIL-18BPc*) and the delivery of anti-IL-17 antibody have eliminated arthritis in a mouse model [44,45].

Delivery of immunosuppressive cytokines

IL-4, -13 and -10 are T-helper (Th)2-derived cytokines known to inhibit IL-1 and TNF production. Several studies in mouse models have demonstrated their beneficial effects on arthritis [27,46–50]. IL-4 is particularly interesting because of its bone and chondrocyte protective action [51–54]. Although IL-10 alone may not be an ideal therapeutic option owing to the finding of decreased IL-10 receptor in RA synovium, its combination with other transgenes may provide benefit [55,56]. IL-13 has been shown to decrease inflammation in an adjuvant-induced rat arthritis model [57]. As with IL-4, IL-13 activity in the joint seems to play a major role in cartilage protection [58].

T- & B-cell inhibition

Inhibition of T-cell activation through the blockade of CD28/B7 interactions has been shown to reduce the severity of several autoimmune diseases. The use of CTLA-4Ig, a fusion protein that binds to the costimulatory molecules B7–1 and B7–2 reduces inflammation in mouse arthritis and in RA patients refractory to TNF inhibitors [59–61]. One-time delivery of CTLA-4Ig in mice using an Ad-vector was found to be as effective as multiple injections of anti-CTLA-4 antibody [62], suggesting that gene therapy could provide long-term efficacy.

Studies showing clinical improvement in RA patients administered rituximab, a chimeric monoclonal antibody against CD20, has renewed the interest in B-cell targeting for RA [63–67]. Belimumab, a humanized monoclonal antibody against BlyS, has also been used but has proven less effective than anti-CD20 therapy in RA [68].

Induction of synovial apoptosis

Pannus formation is central to the destructive features of RA. Pannus results, in part, from the proliferation of synoviocytes. Induction of synoviocyte apoptosis thus offers another target for RA therapy. Fas ligand, which, upon recognition of Fas antigen on target cells, induces apoptosis, effectively induced apoptosis following *ex vivo* Ad delivery to cultured rabbit and RA synovial fibroblasts [69]. Ad delivery of TNF-related apoptosis-inducing ligand, or its recombinant form, also resulted in synovial cell

apoptosis in the rabbit arthritis model [70,71]. *In vivo* delivery of Ad-encoding Fas-associated death-domain protein induced apoptosis of engrafted proliferating human RA synoviocytes in severe combined immunodeficiency mice (SCID) mice [72]. Several cell-cycle regulators have been shown to play an important role in the pathogenesis of aggressive synovial hypertrophy. Both p53 and p53-upregulated modulator of apoptosis induced apoptosis in synoviocytes [73,74]. Ad-mediated overexpression of p21, a cell-cycle regulator controlled by p53, to p53-deficient RA synoviocytes repressed their abnormal invasive properties [75]. Another recent approach involved the targeting of apoptosis promoting transcription factor signal transducer and activator of transcription (STAT)3. Retroviral-mediated gene transfer of a dominant negative mutant of STAT3, termed STAT3-YF, effectively induced apoptosis in synoviocytes [76].

Inhibition of transcription factors

The nuclear factor (NF)-κB pathway is a key intracellular pathway for induction of synovial inflammation [77–80]. Several studies have demonstrated that gene delivery of inhibitors of NF-κB decreased arthritis and inflammatory cytokine production in mouse models. Examples of this include liposome delivery of decoy NF-κB oligodeoxynucleotides and Ad or AAV delivery of a dominant negative form of inhibitor of NF-κB kinase β, a known activator of NF-κB [81–83].

Inhibition of angiogenesis

An early feature of pannus formation is neovascularization. Therefore, the targeting of angiogenesis may act as a means to inhibit early synovial hypertrophy and subsequent cartilage and bony invasion. RV-, LV- and AAV-mediated delivery of angiostatin, endostatin or thrombospondin 1, three antiangiogenic factors, decrease synovial hypertrophy and pannus formation in animal arthritis models [84–88].

Expression of VEGF, a potent stimulator of angiogenesis, correlates with arthritis disease severity [89,90]. The administration of VEGF soluble receptor and the VEGF inhibitor nanogold by Ad vectors effectively reduced arthritis in mice [91,92].

Inhibition of cartilage & bone erosion

The underlying destructive nature and invasiveness of RA synoviocytes are at least in part due to the presence of matrix metalloproteinases (MMPs) [93]. MMP-1 inhibition through retroviral transduction of MMP-1-specific ribozymes and MMP-1 antisense constructs diminished synoviocyte invasiveness [94,95]. A similar reduction in cartilage invasion has been shown with Ad-mediated transfer of tissue inhibitors of MMPs types 1 and 3 in the SCID RA model [96].

Regulation of gene expression

The ability to regulate the level of the therapeutic drug is critical to any successful therapeutic regimen. For gene therapy, this rests in the activation or turning on of select genes during disease flare while being able to turn off expression after disease remission. Drug-inducible promoters provide a targeted method to control transgene expression. One such method developed for this purpose is an antibiotic-inducible system. In this system the antibiotic tetracycline (tet-system) is used to either induce transcription (tet-on) or inhibit transcription (tet-off). The tet-system has been used successfully in rodent arthritis to regulate viral IL-10 expression for up to 2–3 months [97,98].

Inflammation-responsive promoters provide an attractive alternative approach to drug-inducible systems. In these systems, proinflammatory cytokines or transcription factor regulatory elements are used to control gene expression. The promise of such systems has been shown in using the combination of hIL-1β enhancer and IL-6 promoter elements delivered by an Ad vector to regulate transgene expression. Using luciferase as a reporter gene, this system demonstrated the ability to reactivate luciferase expression up to 3 weeks after infection [99]. A second two-component system using complement protein 3 (C3) promoter activation of an HIV promoter for the IL-1Ra gene effectively decreased rodent arthritis after its local delivery (Ad.C3Tat/HIV-IL-Ra) [100]. Recent findings demonstrating the ability of cytokines such as TNF-α, IL-1β and -6 to control AAV transgene expression expands on the use of inflammation itself as a regulatory element in gene therapy [101].

Another method of gene regulation comes in the form of small molecules capable of regulating gene expression once the transgene has been delivered. Proteasome inhibitor (PI) administration has been shown both *in vitro* and *in vivo* to upregulate transgene expression. Interestingly, although the effect is transient, repeated PI administration was able to reinduce gene expression [102].

Table 1. Rheumatoid arthritis gene therapy targets listed with associated vectors and therapeutic transgenes.

Therapeutic approach	Targets	Transgene	Vectors used (Ref.)	Findings
Inflammatory cytokine inhibition	TNF	sTNFR	AAV [34]	Decreased inflammation and bone/cartilage destruction
		sTNFR-IgG	Plasmid [26]	
	IL-1	sIL-1R-Ig	Ad [35]	
		IL-1Ra	Ad [35]	
			RV [39,40]	
	IL-18	IL-18 bp	rAAV [41]	
			Ad [44]	
		IL-18	Ad [43]	Increased bone and cartilage destruction
	IL-17	IL-17	Ad [42]	
Inhibitory cytokines	IL-4	IL-4	Ad [49,52,53]	CIA suppression and cartilage protection
	IL-10	IL-10	AAV [50]	
	IL-13	IL-13	Ad [46–48]	
			RV [56]	
			Plasmid [27]	
			Ad [57,58]	
T-cell inhibition	T cells	CTLA-4Ig	Ad [62]	CIA suppression
Pannus formation	Apoptosis	Fas-L	Ad [69]	FLS apoptosis-sparing chondrocytes
		FADD	Ad [72]	
		TRAIL	Ad [70]	
		p53	Ad [73]	
		PUMA	Plasmid [74]	
		p21	Ad [75,103]	Inhibited FLS migration, CIA suppression
		STAT3	RV [76]	FLS apoptosis
	Angiogenesis	Endostatin	LV [87]	Suppression of TNF-induced arthritis
		Angiostatin	RV [84]	CIA suppression
			HIV [85]	
			AAV [86]	
		sVEGFR	Ad [90]	CIA suppression
Intracellular signaling	NF-κB	IκBα	Ad [78]	Inhibition of NF-κB
		NF-κB decoy ODN	Liposome [81]	
		IKKκBdn	Ad [82]	CIA suppression
			AAV [83]	CIA suppression
Cartilage and bone destruction	MMP	RzMMP1	RV [94]	Decreased FLS invasiveness
		TIMP1 and 3	Ad [96]	Decreased cartilage destruction by FLS
		MMP1 antisense	RV [95]	

AAV: Adeno-associated virus; Ad: Adenovirus; CIA: Collagen-induced arthritis; CTLA: Cytotoxic T-lymphocyte antigen; FADD: Fas-associated death domain; FAS-L: Fas ligand; FLS: Fibroblast-like synoviocytes; IKKk-dn: Inhibitor of NF-kB kinase b; IL1-Ra: IL-1-receptor antagonist; MMP: Matrix metalloproteinase; NF-kB: Nuclear factor-kB; ODN: Oligodeoxynucleotide; PUMA: p-53-upregulated modulator of apoptosis; rAAV: Recombinant AAV; RV: Retrovirus; RzMMP1: MMP-1-specific ribozymes; STAT: Signal transducer and activator of transcription; TIMP: Tissue-specific inhibitors of MMPs; TNFR: TNF receptor; TRAIL: TNF-related apoptosis-inducing ligand; VEGFR: VEGF receptor.

Future perspective

Much progress has been made in the past several years in developing gene therapy as a therapeutic tool for the treatment of arthritis. Areas of future focus will include the targeted delivery of vectors, close regulation of transgene expression, improved vector safety and efficacy and the avoidance of host immune response. Ideally, these systems will specifically target distant sites of inflammation through local or systemic administration. Once

delivered the genes can then be tightly controlled by inflammation-driven promoters. Mechanisms to avoid the host immune response to vectors and their target cells will provide improved duration of gene expression. Finally, the delivery of combinations of genes affecting different targets may induce a more rapid and sustained remission (**Table 1**). Examples would include targeting cytokines at inflammatory lesions while limiting synovial hypertrophy with the induction of apoptosis and inhibition of angiogenesis.

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Executive summary

Advances in gene delivery

- Delivery of therapeutic agents, through viral and nonviral vectors, provides a potential solution to the current limitations in rheumatoid arthritis therapies.
- Development of viral and nonviral vectors capable of avoiding the host immune response improves both transduction and long-term therapeutic efficacy.
- Systemic delivery of immune cells as vectors allows the delivery of transgenes to multiple areas of disease activity, through their innate ability to home to areas of inflammation.

Therapeutic targets for gene therapy in rheumatoid arthritis

- Restoration of the balance between proinflammatory and immunosuppressant cytokines through gene therapy can effectively attenuate arthritis.
- Apoptosis induction and the inhibition of angiogenesis or erosive mediators through gene therapy have prevented aggressive pannus formation.

Regulation of transgene expression

- Use of inflammation and cytokines to drive adeno-associated virus or promoter-specific transgene expression provides an optimal disease regulatory mechanism.
- *In vivo* administration of small molecules such as proteasome inhibitors can enhance transgene expression following viral vector delivery.

Future perspective

- Continued development of low immunogenic vectors able to deliver therapeutic gene combinations targeted at multiple elements involved in arthritis will provide better long-term disease control.

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