Towards gene therapy for rheumatoid arthritis

Abstract

Introduction: Rheumatoid arthritis (RA) is an autoimmune disease of the joint, affecting 0.24% of the global population. Many patients only respond partially, do not respond or develop resistance to current treatments, and the severe systemic complications, including immunosuppression, are unacceptable. Genetic therapies for RA have the potential to improve treatments by targeting delivery to the disease site, enhancing efficacy and avoiding adverse effects.

Areas covered: The route of administration, delivery vector, nucleic acid type and target gene must be carefully selected to develop an effective gene therapy for RA. Drawing from examples of RA gene therapies investigated in animal models and clinical trials, this review discusses how these strategies may be used to translate RA gene therapy into the clinic.

Expert opinion: Existing RA treatments lack specificity to the joint. Genetic delivery systems can include targeting properties, such as disease-responsive promoters or cell-targeting moieties, to overcome this. Non-viral vectors in particular can be engineered easily to possess these properties and, unlike viral vectors, display low immunogenicity. Contrary to current drugs, gene therapy can be delivered intra-articularly, providing sustained levels of the therapeutic. Targeted vectors may also achieve this, but with a single systemic injection, simultaneously delivering the therapeutic to all affected joints.

Article Highlights

- Gene therapy for RA can provide high and prolonged therapeutic gene expression at the disease site, whilst avoiding systemic side effect.
- Intra-articular delivery is possible, but arthritis is polyarticular, so multiple injections would be required. Alternatively, systemic delivery could target all joints with one injection.
- Viral vectors provide adequate efficacy, but safety and immunogenicity are of concern. Non-viral vectors are safer and new formulations may provide the desired efficacy *in vivo*.
- Including cell-targeting properties or inducible promoters into gene delivery vectors may improve potency of the gene therapy, prevent off-target effects and make systemic delivery feasible.

Keywords

Rheumatoid arthritis, gene therapy, viral vectors, non-viral vectors, targeted delivery, intra-articular delivery, systemic delivery

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune condition that is characterised by intra-articular inflammation. It is estimated that the disease affects 0.24% of the global population [1], with predicted levels in developed countries of 0.5-1% [2,3]. Pathological features of the arthritic joint include immune cell infiltration, synovial hyperplasia and increased vascularisation, which lead eventually to the destruction of the cartilage and bone [4]. As a consequence, patients experience significant pain and disability and have an increased rate of mortality [5].

Whilst there is no cure for RA, it can be well-controlled in most cases using non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids, alongside small molecule and biological disease-modifying anti-rheumatic drugs (sm or bDMARDs) [6]. However, despite the improved clinical outcomes observed upon the introduction of bDMARDs, most patients only respond partially and there are still patients that do not respond at all [7,8]. It is estimated that as many as 6% of RA patients have a “bDMARD refractory disease”, meaning disease control was not achieved, following treatment with several different bDMARD [9]. In complications associated with chronic smDMARD treatment, some patients may develop drug resistance, leading to loss of efficacy [10] while in other patients treatments are associated with severe adverse effects resulting from immunosuppression. For example, bDMARDs are associated with an increased risk of serious infection and reactivation of tuberculosis [11]. Immunosuppressive effects are also known with the smDMARDs, further to other systemic complications such as liver damage and bone marrow suppression [12].

Therefore, new therapeutic approaches that improve efficacy and avoid off-target effects are required and so attention has turned to targeting therapies to the joint. With this goal, intra-articular injection has been investigated, but this is inappropriate for many existing drugs due to rapid clearance from the joint. For example, the half-life of various non-steroidal anti-inflammatory drugs (NSAIDs) is just 1-4 hours [13]. Additionally, bDMARDs are cleared from the joint *via* the lymphatic system and removal of macromolecules is enhanced in RA patients [14]. Due to their poor solubility, steroids are an exception to this and are administered as crystalline suspensions that dissolve slowly providing a more prolonged effect [15,16].

Gene therapy presents an alternative approach to treat RA, which has the potential to circumvent the problem of maintaining therapeutic concentrations in the joint by providing the sustained production of therapeutic molecules at the disease site. Furthermore, if delivery of the gene therapy is engineered to target the joint, then systemic delivery could reach all diseased joints with a single injection. Notable progress has been made towards genetic approaches for treatment of RA in animal models and some strategies have reached clinical trial (Table 1). These approaches can be generally grouped into 4 categories based on their target: (1) pro and anti-inflammatory cytokines, (2) genetic synovectomy, (3) angiogenesis and (4) signalling pathways (Figure 1). This review discusses the various features of genetic therapies that are required for their successful translation into the clinic, with particular focus on strategies aiming to target delivery to the joint.

2. Gene therapy molecules

Several genetic strategies exist for RA therapy. Firstly, complementary DNA (cDNA) encoding a therapeutic transgene can be delivered to cells where it is expressed, resulting in the synthesis of a therapeutic protein. The DNA requires a viral or non-viral vector for cellular uptake and may be delivered directly to the patient (*in vivo*) or to cells, which are subsequently transferred to the patient (*ex vivo*). Alternatively, DNA can be delivered as short decoy oligonucleotides, which act to bind and sequester transcription factors, preventing the downstream activation of target gene expression [17]. Antisense RNAs act by binding directly to the mRNA of a target gene to promote cleavage by RNase H and consequently inhibiting target gene expression [18]. RNA interference (RNAi) can also be used to regulate the expression of a target gene post-transcriptionally. RNAs can be introduced as microRNAs, short interfering RNAs (siRNA) or short hairpin RNAs (shRNA), that ultimately result in the activation of RNA-induced silencing complex (RISC) and suppression of a target gene [19].

3. Advantages of RA gene therapy

Since RA primarily affects the joints, intra-articular administration of genetic material has been considered as a means of targeting the joint. By this method, gene therapy could establish a population of cells within the joint that express a therapeutic gene, providing the high concentration of therapeutic at the disease site that is not currently possible with DMARDs. Should expression be stable, just a single injection could provide sustained concentrations of therapeutic in the joint cavity, allowing for long-term treatment. Diffusion of the encoded therapeutic away from the joint would still occur, however the continuous expression of therapeutic would retain high concentrations. Further, the risk of systemic complications would be reduced compared to existing therapies since the dose required for a local gene therapy would be much lower that the whole body approaches currently

used. Therefore, gene therapy approaches in clinical trials and in several experimental models for RA have been delivered intra-articularly (Table 1).

Interestingly, intra-articular delivery of gene therapy may have an additional systemic advantage; a contralateral effect is observed in some animal model studies whereby injection in one paw also induces a clinical improvement in non-injected paws, possibly *via* leukocyte trafficking [20–22]. Therefore, in RA where multiple joints are often affected, a single injection into one joint may also provide therapeutic benefit to other joints without requiring multiple injections.

It is also worth noting that gene delivery vectors can be engineered to target the joint, which may bypass the need for intra-articular injection, but still provide selective therapeutic delivery and expression to all affected joints. For example, promoters have been designed to induce transgene expression specifically at sites of inflammation [23–26] and some non-viral vectors are able to target a certain cell type, such as macrophages, to selectively determine where a therapy is delivered [27]. These targeted delivery systems would have the advantage of being able to target all affected joints with a single systemic injection. Such technologies have the potential to revolutionize genetic approaches to RA therapy, and as such are granted more detail below.

4. Gene delivery strategies

4.1. Ex vivo

The term *ex vivo* gene therapy refers to the genetic modification of cells outside of the body, which, in the case of RA, are then transplanted into the joint of the patient. Since cells are modified *in vitro*, the *ex vivo* approach has several advantages. Notably, the cultured cells can be carefully selected for certain properties. For example, in an *ex vivo* clinical trial delivering interleukin-1 receptor antagonist (IL-1Ra), transduced synovial fibroblasts were screened for replication-competent retroviruses as a quality control step to avoid the risk of insertional mutagenesis, enhancing the safety of the therapy [28]. Additionally, only cells producing IL-1Ra at a rate greater than 30 ng per 10^6 cells in 48 hours were injected, ensuring optimal therapeutic effect. In clinical practice, this type of screening could assure delivery of the correct dose and could be adapted accordingly. Regardless, the *ex vivo* approach was described as costly, laborious and time-consuming [28].

4.2. In vivo

In vivo approaches deliver the gene therapy vector directly to the patient by systemic or intra-articular routes. For RA, focus has mainly been on delivering the therapy *via* intra-articular injection to achieve local expression of a therapeutic transgene in the joint. Approaches delivering nucleic acid therapies

with retroviruses, adenoviruses, adeno-associated viruses (AAV) and non-viral formulations have shown reasonable success in RA animal models (Table 1). The major advantage of the *in vivo* approach is the potential to establish a population of cells in the patient that provide sustained expression of a therapeutic transgene from a single injection, without the need for any complex procedures.

5. Gene delivery vectors

5.1. Viral vectors

Retroviral vectors, such as Molony murine leukaemia onco-retrovirus (MoMLV), are capable of integrating their DNA into the human genome. This property allows for the sustained expression of a transgene however, it also carries the risk of insertional mutagenesis. It was these safety concerns that led to the termination of a study in which synovial cells were transduced *ex vivo* with IL-1Ra in a MoMLV [28,29] as, in a X-linked SCID trial, integration of the retroviral vector into the proto-oncogenes LMO2, BMI1 and/or CCND2 resulted in leukaemias in several patients [30,31]. Additionally, MoMLV vectors can only transduce dividing cells and so their application is limited to *ex vivo* gene therapy [32]. Similarly, lentiviral vectors have demonstrated highly efficient transduction of the synovial lining *in vivo* [33,34], however, safety concerns surrounding integration persist and so lentiviral strategies have not translated into the clinic for RA, so far.

Adenoviral vectors demonstrate effective gene transfer to cells within the synovium [32,35] and so have been used in many experimental *in vivo* studies delivering various transgenes to the joint [20,36,37]. However, as with animal experiments, these vectors induce inflammatory reactions and their direct use in patients is largely precluded because 55% of humans carry antibodies against the adenovirus capsid that can neutralise infection [38,39]. As a result, transduced cells may be ablated by the immune system leading to transient transgene expression. Evidently, this is not appropriate if aiming for long-term transgene expression *in vivo*, however, has been useful for screening target genes in animal models where the animals have no existing immunity.

Adeno-associated viruses (AAV) show more promise for RA gene therapy owing to their reduced toxicity. The virus can stably integrate into chromosome 19 in a site-specific manner, allowing for sustained transgene expression [40], however this requires the viral replication proteins (Rep), which are normally removed from recombinant AAV vectors [41]. Instead, a recombinant AAV genome can be maintained as double-stranded episomal DNA and can reside extra-chromosomally in the nucleus for years, particularly in slowly dividing cells, allowing for long-term transgene expression without the risk of insertional mutagenesis [41,42]. Indeed, delivery of various AAV gene therapies in RA animal

models have demonstrated therapeutic benefit with high transgene expression lasting for several months [43–47]. Further, a clinical trial is currently active (NCT02727764) that delivers an AAV serotype 5 vector encoding IFN-β and expression from the vector in animal models showed high, local transgene expression that could be sustained for 7 weeks [48,49]. However, immunogenicity of AAV vectors remains a problem as humans carry neutralising antibodies to many AAV subtypes as a result of natural exposure and upon administration of AAV vectors, these antibodies can develop, preventing repeated administration [50].

5.2. Non-viral vectors

Non-viral delivery systems eliminate many of the safety concerns associated with viral vectors, including insertional mutagenesis and immunotoxicity. Furthermore, they benefit from reduced cost, ease of access and no restriction on the size of nucleic acid they can deliver. Despite this, non-viral vectors for RA gene therapy have been largely overlooked. This is in part due to reports of low and transient transgene expression and the induction of inflammation in the joints following IL-1Ra DNA delivery with non-viral formulations [32]. However, research into novel non-viral vector systems has progressed in recent years and there is now a wealth of different formulations at a researcher's disposal. Modifications to non-viral vectors, including ionizable lipids [51], peptides [52,53], cell-membrane coating [54], and polymers [27] are improving *in vivo* delivery and may make them more applicable for RA gene therapy in the future. Indeed, in August 2018 the FDA approved a non-viral lipid nanoparticle formulation for the treatment of hereditary transthyretin amyloidosis [55], demonstrating that non-viral systems are able to overcome their historical association with poor potency *in vivo*.

An alternative to delivering DNA for a therapeutic transgene is the use of RNAi. Since they cannot freely pass through the cell membrane, the delivery system is crucial *in vivo* for the RNA to reach the target cell [19]. Non-viral vectors, including cationic liposomes [56], chitosan nanoparticles [57] and poly(lactic-co-glycolic acid) (PLGA) microspheres [56], delivering RNAi therapies for RA have shown success in experimental models. Of particular note is the liposome vector formulated with the cationic lipid RPR209120 and the neutral lipid DOPE. Packaging siRNA, along with carrier DNA, in these liposomes has demonstrated successful silencing of pro-inflammatory cytokines (TNFα, IL-1β, IL-6 and IL-18) [56,58], a lipid inflammatory mediator (phospholipase A2α) [59], and a signal transduction kinase (TAK1) [60]. Interestingly, delivering more than one type of siRNA together (IL-1β, IL-6 and IL-18) in a mouse RA model provided additional protection over individual siRNA therapies [58]. Therefore, non-viral delivery systems could allow for the targeting of multiple pathways involved in

RA pathogenesis simultaneously, providing an increased potency over therapies targeting individual molecules.

Furthermore, the packaging capacity in different hydrophobic and hydrophilic compartments, has enabled delivery of combinations of nucleic acids and small molecule therapeutics simultaneously. In one such approach, siRNA targeting p65 (NF-kB family member), a transcription factor that activates several inflammatory pathways in RA, was first loaded into calcium phosphate nanoparticles, then combined with a smDMARD (methotrexate) and packaged into a liposome [61]. This synergistic method provided excellent efficacy when delivered intravenously to CIA mice with an improved safety profile as compared to delivering free methotrexate alone.

6. Target gene selection

6.1. Pro or anti-inflammatory cytokines

Many RA gene therapy studies have focused on inflammation and restoring the balance of pro and anti-inflammatory molecules. The targeting of pro-inflammatory cytokines for the treatment of RA has already been validated through the development of bDMARDs. For example, there are now several anti-tumour necrosis factor (TNF) biologics approved for the treatment of RA. Accordingly, many genetic approaches have aimed to block TNF activity, but only one therapy, which delivered the equivalent cDNA to the biological drug Etanercept, has progressed to clinical trials [62,63]. In these trials, the cDNA for the soluble human TNF receptor-immunoglobulin Fc fusion gene (TNFR:Fc), demonstrated that gene delivery to the joint was “safe and feasible” [63], and clinical responses were greater in injected groups compared to placebo [62]. However, owing to two serious adverse events (including one fatality) that was since been deemed to be unrelated to the TNFR:Fc therapy, a hold was placed on the trial and there has been no subsequent advancement [62–64].

In experimental studies, TNFα siRNA therapies were investigated with various delivery systems, for example AAV5 [47], cationic liposomes [56], chitosan nanoparticles [57] and PLGA microspheres [65], all of which demonstrated inhibition of TNFα and supressed the progression of RA *in vivo*. Similarly, an antisense 20-base phosphorothioate oligonucleotide that inhibits TNFα reached a phase II clinical trial [66]. The RNA was modified with 2’-O-(2-methoxyethyl) nucleosides in order to improve hybridization to the mRNA, as well as providing protection from cleavage by exonucleases [67]. Pharmacological activity was demonstrated in a Phase II trial for the highest two doses (200 or 400 mg weekly for 40 days) delivered subcutaneously. An improvement in the percentage of patients that achieved a 20% decrease in disease activity by American College of Rheumatology standards (ACR20)

was observed compared to the placebo and this response was sustained at day 169 for >50% subjects [68]. However, after 3 months these responses were inferior to those reported for the biological TNF inhibitors and the trials did not progress further [66–68]. Interestingly, an oral form of the same oligonucleotide was developed with a sodium caprate formulation to enhance intestinal bioavailability, which demonstrated comparable drug tissue concentration to intravenous administration in dogs [69]. Oral administration would provide a preferable alternative delivery route as it would avoid the need for painful injections, increasing patient compliance.

There have also been attempts to inhibit pro-inflammatory cytokines using naturally occurring antagonists. IL-1Ra for instance, is an endogenous antagonist of the IL-1 cell surface receptor and since IL-1 is upregulated in RA, delivery of IL-Ra has been investigated in both *ex vivo* [28,29] and *in vivo* [20,70] approaches. The *ex vivo* approach was the first genetic therapy for RA to reach clinical trial where synovial fibroblast extracted from a patient's joints were transduced with IL-1Ra cDNA using a retroviral vector, before returning the cells to the metacarpophalangeal joints [28]. Two joints received transduced cells, and two control non-transduced cells and 7 days following treatment the synovial tissue was removed and examined for IL-1Ra expression. Elevated IL-1Ra mRNA and protein were found in joints receiving transduced cells, but not in control joints. The therapy progressed to a Phase I trial aiming to determine efficacy, but despite the absence of any adverse events, it was terminated owing to the development of leukaemia in an unconnected SCID trial that used the same retroviral vector backbone [29]. However, patients that did complete the trial reported symptomatic improvement. Similarly, intra-articular delivery of an adenoviral vector encoding IL-18 binding protein (IL-18BP) isoform c, an endogenous neutralizer of pro-inflammatory cytokine IL-18, supressed the development of arthritis in mice with collagen-induced arthritis [71].

In a more unusual approach, Takanashi *et al.* [72] developed a "GeneCream" with a lipid/alcohol base that delivered siRNA to the joint *via* topical administration to the paw of a murine model. Topical administration of therapeutics is an attractive option compared to intra-articular injections. The extracellular matrix cytokine, osteopontin was targeted by an siRNA, as this plays a key role in RA pathogenesis [73]. The study pre-treated type II collagen antibody-induced arthritis (CAIA) mice with the therapy daily for a week before inducing arthritis. Since RA is a chronic disease, where prevention is not an option, it would be important to test this formulation in a model with established disease more akin patients with RA. .

It is also possible to modulate inflammation using regulatory anti-inflammatory cytokines, restoring the balance of the cytokine profile. For example, IL-13 can diminish the production of proinflammatory cytokines in RA synovial tissue [74] and in CIA mouse models IL-4 [75] and IL-10 [26,76] were shown

to have a protective effect on bone and cartilage. More recently, a gene therapy encoding interferon-β (IFN-β) cDNA was delivered using an AAV5 vector (AAV5.IFN-β) [25,48,49]. IFN-β is an immunomodulatory cytokine with the ability to downregulate pro-inflammatory or upregulate anti-inflammatory cytokines, as well as slow cartilage degradation [25,77]. In this therapy, IFN-β is under the control of an inflammation-inducible promoter, responding to NF-κB in the inflammatory environment. Intra-articular injection in the adjuvant arthritis rat model showed local IFN-β expression that reduced paw swelling and exerted a chondro-protective effect [25]. Further preclinical studies have shown promise and a Phase I clinical trial is currently ongoing [48,49].

6.2. Genetic synovectomy

Fibroblast-like synoviocytes (FLS) are key effector cells in RA pathophysiology. They become hyperplastic and express an array of cytokines, chemokines and growth factors, as well as playing an active role in cartilage degradation [78]. Accordingly, the synovium of an inflamed joint can be surgically, chemically or radiologically removed to alleviate pain and inflammation [79]. However, this is an invasive procedure and therefore, the delivery of cytocidal genes, which requires only transient expression of the transgene, to reduce the hyperplastic synovium has been considered as an alternative. The first of these approaches delivered thymidine kinase, an enzyme of the herpes simplex virus that can metabolise the antiviral drug ganciclovir [36]. When phosphorylated by thymidine kinase, the metabolised drug induces apoptosis *via* inhibition of DNA polymerase. Intra-articular injection of thymidine kinase in an adenoviral vector, followed by intravenous injection of ganciclovir caused extensive apoptosis of the synovium of rhesus monkeys with CIA, demonstrating the feasibility of synovial ablation.

Other strategies have taken advantage of molecules already highly expressed in the RA synovium. For example, Fas is a death receptor located on the cell surface that can induce cell death upon interaction with Fas ligand (FasL) [80]. Expression of Fas in the RA synovium is considerable, however, little FasL expression is observed [81,82]. Therefore, delivery of FasL has been investigated as a method to induce apoptosis in the synovium of a rabbit IL-1β-induced model [81] and the human RA synovium grafted onto SCID mice [83]. In both studies, significant reduction in the size of the synovium was observed and the viability of cartilage did not appear to be compromised.

Overexpression of p53 in the joint has also shown promise [84]. p53 is a tumor suppressor involved in cell cycle arrest and progression towards apoptosis [85]. Interestingly, high levels of p53 are observed in the RA synovium, yet apoptosis occurs slowly [86]. Mutations in p53 have been associated with RA and some of these mutations have been found to inactivate the molecule, explaining the lack of cell

death [87]. Delivery of the p53 gene using an adenoviral vector in an IL-1β induced arthritis rabbit model promoted significant apoptosis and reduced leukocyte infiltration [84].

6.3. Targeting angiogenesis

Angiogenesis plays a pivotal role in the progression of RA, enabling inflammatory cell infiltration and the increased production of inflammatory mediators that come alongside them [88]. Hence, delivery of genetic material that can inhibit angiogenesis is another possible direction for RA genetic therapies. One regulator of angiogenesis is vascular endothelial growth factor (VEGF), which contains a hypoxia-response element (HRE) in its promoter region [83] and, as a result ,is upregulated in response to the hypoxic environment of the inflamed joint [88]. In fact, a correlation between serum VEGF levels and the severity of RA has been demonstrated [89]. Therefore, when VEGF was inhibited by expressing the secreted extracellular domain of the Flt-1 VEGF receptor with an adenoviral vector in CIA mice, disease severity was suppressed, suggesting blocking VEGF could be an option for RA therapy [90]. Similarly, the endogenous angiostatic factor, angiostatin, was delivered to the knee joints of CIA mice using an *ex vivo* approach that transduced NIH3T3 cells with a retroviral vector [91]. Angiogenesis was inhibited in treated mice and the progression of arthritis was reduced.

Zhou et al. [46] also aimed to investigate VEGF as a target for RA therapy, but in the context of the lymphatic system. Interestingly, lymph-angiogenesis also occurs in RA, but this is not accompanied by an increase in lymphatic flow [92]. By delivering VEGF-C with an AAV vector, Zhou *et al.* [46] were able to improve the lymphatic drainage from the joints of the TNF transgenic mouse RA model and demonstrated that VEGF-C could reduce inflammation and bone and cartilage damage [46].

6.4. Targeting signalling pathways

Various signalling pathways become highly activated in RA. The NF-κB pathway, for instance, plays a major role in the regulation of proinflammatory cytokines, making it an attractive target for RA intervention [78]. Decoy oligonucleotides that can sequester endogenous NF-κB have been investigated as a genetic approach to NF-κB inhibition [88]. Unfortunately, the use of decoy oligonucleotides is limited by their poor pharmacokinetics, largely due to poor cellular uptake and their deactivation by nucleases [17]. Consequently, efforts have been made to protect NF-κB decoy oligonucleotides and improve their delivery for the treatment of various inflammatory conditions (reviewed in detail by Farahmand et al. [17]). One such approach delivered hemagglutinating virus of Japan (HJV) liposomes loaded with the NF-κB decoy oligodeoxynucleotides to the collagen-induced arthritis (CIA) rat model [93]. The treatment provoked a reduction in paw swelling and the levels of TNFα and IL-1β. Another method used a N-trimethyl chitosan-polysialic acid (PSA-TMC) nanoparticle

that was coated by NF-κB decoy oligonucleotides [94]. The delivery system improved uptake of the oligonucleotides and reduced the expression of inflammatory cytokines in primary synovial cells derived from RA patients and human FLS.

The MAPK signalling cascade is also extremely active in RA with phosphorylated MAPKs responsible for the activation of various transcription factors [78]. Ras is a GTPase that leads to the activation of MAPK pathways and interestingly it is upregulated in RA FLS [78,95]. Targeting the Ras pathway by delivering a dominant negative form of the RAS gene (AxRasDN) with an adenovirus vector reduced the production of proinflammatory cytokines and the proliferation of RA FLS [95]. Furthermore, AxRasDN suppressed inflammation and bone erosion in rat RA models. This highlights the potential of targeting MAPK pathways for the treatment of RA.

6.5. Micro RNAs

Short, single-stranded non-coding RNAs, known as micro RNAs (miRNAs) are involved in the regulation of protein expression. They usually bind multiple mRNAs to either inhibit translation, or promote mRNA degradation [96]. Expression of miRNAs is abnormal in RA, for example the aggressive phenotype of activated FLS is, amongst other things, controlled by changes in miRNAs [96,97]. As such, various miRNAs have been suggested as targets for RA therapies (reviewed in detail by Vicente et al. [97]). Since a single miRNA may control several genes, these therapies would have the advantage of controlling the disease in numerous downstream pathways simultaneously. As an example of how this may work therapeutically, the microRNA miR-155 could both reduce the expression of MMP-3 and the proliferation of RA FLS *in vitro* [98]. Therefore, miR-155 could provide protection from inflammation and cartilage degradation in RA.

7. Targeted gene delivery vectors

Whilst intra-articular delivery results in production of the therapy in the synovial tissue, clearance away from the joint may still be a problem in terms of efficacy and systemic side effects and multiple injections may be required to treat all affected joints. Instead, some approaches have aimed to ensure gene expression is specific to arthritic joints to improve both gene delivery and safety. The goal of these methods is to design vectors that target gene delivery to the inflamed synovium, such that a single systemic injection results in localised therapeutic expression simultaneously in all arthritic joints. This has been achieved by two general method: (1) designing responsive promoters that induce

transgene expression only at sites of inflammation, (2) engineering non-viral vectors with tropism for a certain cell type.

7.1. Disease-responsive vectors

The concept of 'physiologically-responsive' expression promises to enhance the specificity of gene therapies by restricting therapeutic gene expression to the site of disease. Expression of the therapeutic gene is controlled by the disease course itself. In the context of RA, disease-responsive gene therapies would be particularly useful as they would provide increased anti-inflammatory effects during a disease flare, but would be less active or inactive during periods of disease remission. Various endogenous promoters have been adopted experimentally for this purpose. For example, in murine models of RA disease-responsive expression of anti-inflammatory cytokine IL-10 has been driven by, E-selectin [23], Saa3 [24] and MMP13 [24] promoters and an IL-6 promoter fused to an IL-1 enhancer [26,99]. Additionally, IL-10 expression was also inducible under a CXCL10 promoter in stimulated primary synoviocytes derived from RA patients and this treatment reduced the expression of inflammatory cytokines [100].

Alternatively, disease-responsive synthetic promoters have been designed with transcription factor binding sites (TFBS), such as NF-κB [101,102] and HIF-1α (hypoxia-responsive) [103], upstream of a minimal promoter. These have the advantage over endogenous promoters in that the selected TFBS are tailored to the disease in question. Endogenous promoters may possess unrelated TFBS that could cause aberrant promoter activation. Such synthetic responsive promoters are already being incorporated into gene therapy approaches for RA. For example, as described earlier an AAV5 vector carrying an NF-κB responsive promoter regulating IFN-β expression has recently entered clinical trials [25,48]. This promoter contains six repeats of the NF-κB binding motif and levels of IFN-β mRNA and protein were much greater under the control of this promoter, as compared to a conventional CMV promoter [25]. Finally, the concept of multi-responsive synthetic promoters that are activated by a combination of various disease-related transcription factors has also been considered, the benefits being that therapeutic gene expression more closely parallels the course of the disease and could be tailored to an individual disease [104].

7.2. Targeting cells in the joint

Direct delivery of a gene therapy to cells involved in RA, such as macrophages or FLS, would selectively improve the targeting of treatment to the disease site, which could prevent off-target effects caused by gene delivery to cell-types not involved in RA pathophysiology. To this end, non-viral vectors, such as chitosan [105] and lipid-based nanoparticles [61], have been conjugated to folic acid to deliver

siRNA to target activated macrophages, which overexpress the folate receptor on their surface. In both studies, the uptake of the nanoparticles was increased in activated macrophages as compared to inactivated *in vitro*, and Yang et al. [105] demonstrated that the chitosan/folate vector could accumulate at sites of inflammation *in vivo*. Furthermore, Song et al. [27] have described a "polymer-lipidoid hybrid nanoparticle" named FS14-NP, which has also shown selectivity for macrophages. FS14-NP/siRNA complexes accumulated in arthritic joints and, when IL-1β siRNA was delivered, significant improvements in arthritis score and paw swelling, as well as reduction in proinflammatory cytokines, were observed in CAIA mice.

The idea of targeting therapeutics directly to cells of the inflamed joint isn't unique to gene therapy. For example, recently PLGA nanoparticles coated with platelet [106] or macrophage-derived microvesicle [107] membrane have been shown to target and accumulate at the inflamed joint *in vivo*. Additionally, neutrophil membrane-coated PLGA nanoparticles have shown affinity for inflamed cells of the RA joint and owing to intrinsic proteins of the neutrophil membrane, could lessen disease severity in CIA mice without the need for delivering a drug/gene cargo [54]. Such membrane-coated non-viral systems are yet to be reported for RA gene therapy, but may provide another method to hone delivery to the inflamed joint.

Finally, several peptides with the ability to target the synovium have been described and investigated for targeted therapeutic delivery. For example, Yang *et al.* (2011) [108] describe two peptides with specificity for endothelial cells of the inflamed synovium, whilst Wythe *et al.* (2013) [109] showed that coupling the synovial targeting peptide SyETP to the anti-inflammatory cytokine IL-4 could increase the cytokine's accumulation in human RA synovial tissue SCID model and lengthened its half-life. Similarly, Mi et al. [110] identified the HAP-1 peptide by screening a rabbit synoviocyte cell line with an M13 phage display library. HAP-1 demonstrated the ability to selectively target synovial fibroblasts *in vitro* and *in vivo* and when coupled to a pro-apoptotic peptide could specifically drive synovial cell death. If targeting peptides can be internalised, it may be possible to utilise these in gene therapy vectors to specifically deliver the DNA/RNA cargo to the RA joint.

7.3. What cells to target?

Historically, administration of genetic therapies by intra-articular injection has aimed to target gene delivery to the synovium. Considering that the fibroblast and macrophage-like synoviocytes that reside there are heavily involved in disease development and progression, this seems intuitive. However, depending on the target gene and nucleic acid, this may not be necessary. For example, any cell local to the joint would be an appropriate target for a gene therapy encoding a secreted inhibitor

of inflammation. In fact, studies have found that intra-articular delivery to the synovium does not sustain transgene expression for more than a few weeks [22,91,93,111]. Gouze *et al*. [111] report that FLS have a high rate of turnover that would eventually lead to the loss of the therapy, particularly in RA where the aim of the therapy is to reduce synovial hyperplasia in the first place. Instead, they suggest that targeting cells in the surrounding connective tissues, such as ligaments, tendon and capsule, to produce a secretable transgene may provide more stable, long-term expression. On the contrary, this would not be appropriate for RNAi, anti-sense RNA or decoy oligonucleotide therapies, which would need to be directly delivered to the cell type in which their target gene is actively being expressed, such as FLS or inflammatory cells. Furthermore, synovectomy-based approaches would be improved by specifically targeting the synovium as it would reduce the risk of inducing apoptosis in other cell types. Ultimately, to provide a therapy with optimal efficacy, but lacking adverse effects, targeting would need to be tailored to both the nucleic acid strategy and the target gene (Figure 1).

8. Conclusion

Many genetic approaches to RA therapy using different vectors and targeting different genes have been investigated and their efficacy has been extensively demonstrated in animal models, and some in human trials. To overcome problems faced with existing therapeutic strategies, intra-articular injection of genetic therapies promises to provide local and sustained therapeutic concentrations at the joint. However, no approach has yet demonstrated both the required efficacy and safety in clinical trials. More studies that aim to refine gene delivery for RA, such as regulated transgene expression and cell-type specific vectors, are required. With the advantages of improving delivery of therapeutics to the site of the disease and avoiding non-specific delivery and associated adverse effects, it is likely that such targeted delivery systems will continue to develop in the near future. This is especially true for non-viral delivery vectors that can be easily engineered to possess targeting properties.

9. Expert Opinion

Whilst genetic therapies for RA have the potential to overcome many of the obstacles experienced by current treatments, several barriers remain before their successful translation into the clinic. Firstly, since RA is a chronic condition lasting for decades, an appropriate vector must be found that results in a prolonged therapeutic effect. Additionally, the vector must not be immunogenic as repeated administration will be necessary for long-term treatment. Whilst viral vectors can provide the

sustained transgene expression required, they all demonstrate immunogenicity to some extent [38,39,50]. Non-viral vectors on the other hand, show no immunogenicity, but historically have not provided the desired efficacy [32]. However, many new non-viral formulations are in development and are starting to make their way into the clinic. With respect to RA, non-viral vectors delivering either siRNA or plasmid DNA have already shown efficient treatment of disease in arthritis animal models (Table 1). Another major advantage of non-viral vectors is that they can be easily modified to possess targeting properties, for example the conjugation of chitosan or lipid nanoparticles with folic acid to target macrophages [61,105]. Investigation of novel approaches that include such targeting components, or are even coated with cell membrane, are exciting fields of study, with the potential to provide a delivery system that has the required potency at the disease site, as well as an improved safety profile. Finally, since there is no risk of viral integration with non-viral formulations, this type of therapy may overcome the historical safety concerns that deemed gene therapy too hazardous for a non-lethal disease such as RA.

The route of administration of the genetic therapies must also be carefully considered. Local delivery via intra-articular injection has demonstrated efficient transgene expression at the joint. However, RA is normally polyarticular and therefore, injections into all affected joints would likely be required making for invasive treatment and complicated dosing. Preferably, one systemic injection would deliver the gene therapy to all affected joints simultaneously and whilst this may need to be repeated for long-term treatment, it is a relatively simple procedure compared to the intra-articular alternative. Disease-responsive promoters and cell or tissue type-specific non-viral vectors may provide approaches for achieving this type of delivery in the future as they allow for localised expression with systemic delivery. Exactly where to target the therapy remains in question, but will need to be tailored to the transgene, vector and nucleic acid type, and further investigation is required in this area.

Finally, the selection of transgene will be important and an overwhelming repertoire have already been investigated for RA gene therapy in both animal models and some in human trials (Table 1). Considering that many of these transgenes will result in the expression of the same therapeutic molecule, or target the same pathway as existing bDMARDs, responsiveness of individual patients to the gene therapy could be a major issue considering 6% of patients receiving bDMARD treatment are non-responsive to multiple bDMARDs [9]. Thus, the question of responsiveness to genetic therapies remains, however with the possibility of providing greater therapeutic concentrations at the joint, but with reduced systemic concentrations, gene therapy approaches may still provide better clinical outcomes than existing drugs. As our understanding of RA and its treatment improves it may become

clear which pathways are the best targets. In addition, it may be that a 'one size fits all' approach is not suitable for RA and the target pathway may need to be adjusted to an individual patient's disease.

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