Gene therapy in autoimmune disease
Ingo H Tarner* and C Garrison Fathman†

Recent work on gene therapies for autoimmune disease has continued to provide insight into the pathogenesis of autoimmunity. Reliable, effective and targeted gene therapy applications have been achieved by using transduced dendritic cells and antigen-specific T cells as delivery vehicles. Bioluminescence imaging has been implemented to visualize cell trafficking and homing in vivo. As a first step into human gene therapy, a phase I clinical trial for assessing the feasibility and safety of gene transfer has been completed in a group of rheumatoid arthritis patients.

Current strategies in gene therapy of autoimmune disease
Gene therapy is generally defined as the introduction of DNA into a host cell for therapeutic purposes. DNA insertion into host cells followed by expression of the gene product(s) of interest is intended to achieve one of three effects. First, the targeting of a known genetic defect. In this case, the inserted gene replaces or compensates for a defective or missing gene in the host. Second, delivery of immune-modulating molecules. In this case, the inserted gene encodes anti-inflammatory molecules, for example, cytokines such as IL-4, IL-10 or TGF-β, cytokine antagonists such as IL-1R (IL-1 receptor) antagonist or IL-12 p40, soluble cytokine-receptors or blocking antibodies such as TNF antagonists, chemokine antagonists and complement antagonists, all of which antagonize pro-inflammatory autoimmune reactions. Third, interference with signaling processes involved in autoimmune reactions, for example, TCR signaling and co-stimulation or apoptosis pathways.

Various approaches are used to introduce DNA into host cells, including naked DNA, DNA complexed with liposomes, and various viral vectors. Viral vectors can be either injected systemically or locally into the host tissues, or can be used to transfect or transduce host cells in vitro, which are then adoptively transferred and serve as delivery vehicles, such as T cells [1,2,3••], fibroblasts [4•,5,6] and dendritic cells (DCs) [7,8••,9••].

Targeting known gene defects
The genetic basis of most autoimmune diseases is polygenic. Thus, targeting a single missing or defective gene is generally not applicable. The only recent studies along these lines aim at symptomatic treatment by replacing missing or reduced gene products, such as insulin in diabetes, even though the lack of insulin is not due to a single gene defect. Two interesting studies report remarkable success in insulin replacement by gene therapy. Falqui et al. [10*] transduced fibroblasts with a retroviral construct encoding a genetically modified human pro-insulin that is cleavable into insulin in non-β cells. Transplantation of these fibroblasts into diabetic mice reverted hyperglycemia. By contrast, Lee et al. [11••] used a single-chain insulin analog (SIA) instead of pro-insulin to treat streptozotocin-induced diabetes in rats and autoimmune diabetes in non-obese diabetic mice. The SIA was coded by a recombinant adeno-associated virus vector (rAAV) with a rat L-type pyruvate kinase (LPK) gene promoter, which regulates SIA expression in response to blood glucose levels. Injection of rAAV–LPK–SIA into the portal vein led to integration of SIA DNA into the chromosomal DNA of hepatocytes. SIA expression under control of the LPK promoter was found to be controlled by blood glucose levels.

Introduction
Autoimmune diseases are a large and diverse group of disorders characterized by inappropriate responses of the immune system against self-tissue. In general, autoimmune diseases are systemic disorders that affect the whole body; however, they can be divided into separate entities based on the predominant affection of target organs or tissues. The most common entities are rheumatoid arthritis, insulin-dependent diabetes mellitus and multiple sclerosis.

As the exact pathogenesis of these disorders remains largely elusive, conventional treatment is limited to relatively nonspecific suppression of the immune system, which is itself associated with severe, adverse side-effects. Gene therapy approaches appear to hold promise for more specific, targeted treatments, which have evolved from an increased understanding of the pathophysiological mechanisms of autoimmune disease. Specific aims for gene therapy include the development of safe and specific techniques for gene or gene product delivery with minimal adverse effects.

Here we review currently used gene therapy approaches, as well as recent advances in strategies for gene therapy of autoimmune disease.

Abbreviations
AIA = adjuvant-induced arthritis
CIA = collagen-induced arthritis
DC = dendritic cell
EAE = experimental autoimmune encephalomyelitis
FasL = Fas ligand
IL-1R = IL-1 receptor
LPK = L-type pyruvate kinase
SIA = single-chain insulin analog

Addresses
Stanford University School of Medicine, Department of Medicine, Division of Immunology and Rheumatology, CCSR Building, Room 2225, 300 Pasteur Drive, Stanford, CA 94305-5166, USA
*e-mail: ingo.tarner@stanford.edu
†e-mail: cfathman@stanford.edu
Correspondence: C Garrison Fathman

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and resulted in long-term remission of diabetes in both animal models tested. Notably, this treatment did not have any adverse effects on the hepatocytes.

These findings are very encouraging with regard to the symptomatic treatment of human autoimmune diabetes. In particular, gene therapy that can facilitate the secretion of either insulin or an insulin-analog according to the blood glucose levels would be an attractive alternative to the current insulin replacement techniques of subcutaneous injections.

Introduction of immune-modulating molecules

The delivery of immune-modulating molecules by various means represents the most commonly investigated strategy of gene therapy in autoimmune diseases. Because cytokines are known to play a pivotal role in the pathogenesis of autoimmune disease, there has been extensive study of the application of cytokines with immuno-regulatory properties, primarily IL-4 and IL-10, and inhibitors of pro-inflammatory cytokines, such as anti-TNF antibodies, soluble receptors, IFN-γR and the IL-12R antagonist IL-12p40.

The anti-inflammatory cytokines IL-4 [12–14,15*], IL-4–IgG1 chimeric protein [16,17], IL-10 [18,19], Epstein–Barr virus-encoded vIL-10 [20–22] and IL-13 [23] — all used extensively in previous studies — have been confirmed recently to be beneficial in disease prevention [9**,12,14,15,19–23], as well as in therapeutic studies [8**,13,14,15*,16,20–23], multiple sclerosis [13,17], diabetes [19] and autoimmune thyroiditis [18]. Soluble TNFR molecules [6,22,24], IFN-γR–IgG fusion molecules [25,26] and the IL-12R antagonist IL-12p40 [3**,27] have been used successfully in various studies in order to antagonize the pro-inflammatory cytokines TNF, IFN-γ and IL-12.

Immuno-modulation of established disease

In general, gene therapy applications of single cytokines or cytokine antagonists have been very successful for preventing autoimmune disease. But because it is impossible to predict the onset of autoimmune disease in humans, it is clinically more relevant to study the therapy of established disease. Only a few studies report successful treatment of established disease in the various models, and only a few groups have tested combination therapy.

Such ‘treatment’ studies using single cytokines have yielded variable results. For example, Martino et al. [13] have been able to treat experimental autoimmune encephalomyelitis (EAE) by intra-thecal injection of HSV-1-derived vectors encoding IL-4. Likewise, in rat adjuvant-induced arthritis (AIA) and murine collagen-induced arthritis (CIA), the application of IL-4 has been reported to be therapeutic [14,15*].

Woods et al. [15*] have shown that injecting adenovirus encoding rat IL-4 into inflamed AIA ankles reduces the severity of clinical arthritis and the levels of pro-inflammatory cytokines and chemokines in ankle homogenates. Moreover, Kim et al. [8**,14] have reported a reduction of inflammation in CIA after intra-articular injection of recombinant adenovirus encoding murine IL-4, and also almost complete disease suppression using intravenous adoptive transfer of DCs expressing IL-4 after adenoviral gene transfer.

By contrast, in studies on CIA by Wim van den Berg’s group, systemic administration of IL-4 suppressed active disease and showed a cartilage-protective effect [28], whereas no improvement of clinical disease was noted when IL-4 was administered by intra-articular injection of recombinant human type 5 adenovirus encoding IL-4 [29]. Nevertheless, van den Berg’s group observed a chondro- and osteo-protective effect.

IL-10 has been used successfully in the treatment of murine autoimmune diabetes. Batteux et al. [18] were able to abolish lymphocytic thyroid infiltration and reduce IFN-γ levels as well as the anti-thyroglobulin antibody response, by surgical instillation of liposome and poly-L-lysine-complexed DNA encoding IL-10 into inflamed murine thyroid glands. In established murine CIA, however, adenoviral gene transfer of viral IL-10 had only minimal effect, despite effective disease prevention, when administered before the onset of clinical disease [22]. In the same study, Kim et al. [22] reported that adenovirus-encoded divalent human p55 soluble TNFR–Ig/murine IgG1 fusion protein had no effect on murine CIA. In EAE, by contrast, the dimeric p75 TNFR, which was produced by adoptively transferred immortalized fibroblasts after retroviral transduction, ameliorated both acute and relapsing disease [6].

The reasons for these discrepancies are not entirely clear yet, and this issue has not been addressed adequately. It is conceivable that, in a multifactorial and complex disease process such as autoimmunity, the application of a single cytokine or cytokine inhibitor alone is not sufficient to control the inflammatory cascade, or is only useful early on in the inductive autoimmune processes.

Preventative versus therapeutic effects

Woods et al. [15*] have compared the effects of intra-articular adenoviral delivery of IL-4 in rat AIA before (preventative approach) and after (therapeutic approach) disease onset and have found some remarkable differences. In both preventative and therapeutic approaches, adenoviral delivery of IL-4 reduced clinical disease severity and inhibited weight loss. But although preventative administration of IL-4 led to improved bone integrity on X-ray scoring and reduced the number of monocytes, polymorphonuclear cells and blood vessels on histopathological joint analysis, therapeutic administration did not significantly improve bone erosions compared with PBS treatment and did not affect the cellular infiltrate and number of blood vessels.

Furthermore, preventative IL-4 administration reduced levels of the pro-inflammatory chemokine MCP-1 in rat ankle homogenates, whereas therapeutic administration of
IL-4 had no effect on MCP-1 levels but significantly reduced the levels of IL-1, TNF, MIP-2 and RANTES. These results point to different mechanisms of action at different stages of the disease. Woods et al. [15•] also describe a pro-inflammatory effect of adenovirally produced IL-4 at low adenovirus doses (5 × 10^6 plaque-forming units [PFU]) compared with an anti-inflammatory effect at higher doses (1 × 10^8 PFU) when used in a preventative fashion, suggesting that there may be a dose dependence that could vary considerably depending on the disease model, vector type and anti-inflammatory molecule used.

**Mode of administration**

In addition to the differing effects of the same molecule according to the disease stage and the dose, the mode of administration also seems to influence efficacy. As mentioned above, systemic administration of IL-4 suppressed active CIA [28], whereas intra-articular injection of IL-4-encoding recombinant human type 5 adenovirus [29] did not improve the disease. Intravenous injection of adenovirus encoding IL-4 was found to reduce the severity of early stage CIA, and peri-articular injection of adenovirus encoding IL-4 resulted in reduced severity of established disease [14]. Kim et al. [8••] found that even better treatment could be achieved by intravenous adoptive transfer of DCs that express IL-4 after adenoviral infection. Similarly, Morita et al. [9••] reported that IL-4-transduced DCs that were adoptively transferred before disease onset reduced the incidence and severity of murine CIA, whereas IL-4 delivery by retrovirally transduced T cells and NIH 3T3 cells had no effect.

**Adverse effects**

Immunomodulation with cytokines and cytokine inhibitors can have unexpected, adverse effects, which abolish their beneficial effects. For example, Quattrocchi et al. [24] reported an initial beneficial effect followed by disease rebound in murine CIA, despite continued treatment with both adenovirus-mediated gene delivery and direct injection of a dimeric chimeric human p55 TNFR-IgG fusion protein. The authors identified an increased antibody response to type II collagen and increased agonistic antibodies to TNFR as potential causes for the loss of the initial beneficial effect.

A study by Chen et al. [30] raises the suspicion that a vigorous shift in the cytokine balance towards Th2 responses can be deleterious instead of beneficial. In their study, the authors bred mice that are transgenic for an IL-2Rβ–IL-4Rα chimeric cytokine receptor that transduces IL-4-specific signals in response to IL-2 binding, thereby enhancing Th2 responses including increased levels of IL-4, IL-5 and IL-10 as well as increased levels of collagen-specific IgG3. When CIA was induced in these animals, earlier onset and a greater incidence and severity of disease were found instead of protection against disease.

**Combination therapy**

As a means to reduce unwanted effects and to improve the therapeutic effectiveness of using cytokines and cytokine inhibitors, combination therapy seems to hold promise, as suggested by an adenoviral gene therapy study in CIA [22]. A combination of viral IL-10 and soluble TNFR was effective in prevention and therapy, whereas either agent alone had limited or no effect, respectively. A combination of different strategies can also be successful, for example, combining the anti-inflammatory effects of IL-4 and the pro-apoptotic effect of Fas ligand (FasL) [31].

**Interference with signaling processes involved in autoimmunity**

Whereas studying the effects of cytokines and cytokine inhibitors has been the main focus of gene therapy in autoimmune research in the past, more and more studies are looking at alternatives for modulating autoimmune processes. Among these approaches are induction of apoptosis in pro-inflammatory cells, prevention of apoptosis in tissue cells, interference with TCR signaling, tolerance induction in immune effector cells, and various other strategies.

Apoptosis of pro-inflammatory, autoantigen-specific, organ-infiltrating lymphocytes, and thus induction of tolerance to autoantigens in autoimmune disease, can be achieved by expressing FasL on antigen-presenting cells such as macrophages [32,33], by the direct injection of FasL-encoding adenoviral vector into inflamed tissues, such as the salivary glands in a murine model of Sjögren’s syndrome [34], or by local injection of plasmid DNA that leads to transfection of and FasL expression on, for example, thyroid follicular cells in a murine model of experimental autoimmune thyroiditis [18].

Guery et al. [31] have reported a beneficial effect in murine CIA from apoptosis induction in neutrophils after FasL expression on genetically engineered Chinese hamster ovary cells. Other methods to induce apoptosis in pro-inflammatory cells include facilitating TNF-induced apoptosis through either preventing nuclear translocation of the nuclear factor NF-κB or inhibiting the TNF-induced upregulation of X-linked inhibitor of apoptosis [35]. Because NF-κB also regulates the expression of pro-inflammatory cytokines such as IL-1 and TNF, interference with NF-κB activity could be therapeutic through both apoptosis induction and downregulation of pro-inflammatory cytokines. This has been demonstrated by Tomita et al. [36], who took the interesting approach of injecting NF-κB-binding decoy oligodeoxynucleotides intra-articularly in rat CIA. A particularly interesting strategy has been described by Rabinovich et al. [4•]. After adaptively transferring fibroblasts expressing recombinant Galectin-1 — a β-galactosidase-binding protein — into CIA mice, these authors observed a pronounced therapeutic effect and increased susceptibility to antigen-induced apoptosis in lymph node cells taken from the treated mice.

Instead of inducing apoptosis in pro-inflammatory cells, some groups have tried preventing the apoptosis of tissue cells. Apoptotic destruction of β cells in the pancreatic
islets of Langerhans, for example, has a role in the development of autoimmune diabetes. This destructive mechanism has been successfully blocked by adenovirus-mediated overexpression of the anti-apoptotic gene A20 [37,38], or expression of the anti-apoptotic gene bcl-2 using a replication-defective herpes simplex virus vector [39].

TCR signaling is another attractive target for gene therapy of autoimmune diseases. T-cell activation requires two signals: engagement of the TCR with its specific antigen presented by a MHC class II molecule on an antigen-presenting cell (signal 1); and a co-stimulatory signal (signal 2) such as interaction between CD80/CD86 and CD28, which leads to T-cell activation and proliferation. Interaction between CTLA-4 and CD80/CD86 can, however, down-regulate activated T cells [40]. Intravenous administration of adenoviral vectors encoding a CTLA-4-Ig fusion protein has been successfully used to ameliorate EAE [41], nephritis in a murine lupus model [42] and murine CIA [43].

In the context of TCR signaling, the induction of T-cell tolerance is also of interest. For example, retroviral transduction of antigen-presenting B cells to present autoantigens or immunogenic epitopes has been shown to induce tolerance to the disease-causing autoantigen, and to induce protection against EAE [44] and experimental autoimmune uveitis [45]. Among the possible mechanisms underlying this phenomenon are induction of T-cell anergy by resting B cells providing signal 1 without signal 2 [44], and delivery of a tolerogenic signal through, at least in part, CTLA-4 [45,46].

Miscellaneous strategies for the gene therapy of autoimmune disease include inhibiting the complement system by retrovirus-mediated expression of soluble complement receptor 1 [5], downregulating molecules involved in disease pathogenesis by injection of antisense oligonucleotides [47], administering anti-inflammatory molecules other than cytokines or cytokine inhibitors, such as the reactive oxygen species scavenger catalase [48], or mediating tissue repair through expression of a regenerative growth factor (platelet-derived growth factor A) in EAE [49].

Recent advances in gene therapy of autoimmune disease

Despite a wealth of different approaches to gene therapy, as outlined above, there has been no major breakthrough. Nevertheless, there are some promising strategies under investigation. An ideal gene therapy approach should provide targeted and reliable delivery of the therapeutic gene(s) and its product(s), coupled with regulated and long-term gene expression dependent on the activity of the disease.

Targeted, local delivery of genes and gene products has been achieved using antigen-specific T cells [1,3*,27,50,51**] or T-cell hybridomas [3**] as site-specific gene delivery vehicles. In the past, effective use of antigen-specific T cells was impeded by a low transduction efficiency. Our group has developed a novel retroviral construct that contains an internal ribosomal entry site (IRES). This bicistronic expression vector allows co-expression of two genes, the gene of interest and a fluorescent-marker-encoding gene, on one mRNA. Using our novel construct we have been able to stably and durably transduce primary T cells and T-cell hybridomas with up to 80% transduction efficiency [3*,51**]. Moreover, by using bioluminescence imaging, which allows repeated injections of the substrate, luciferin. Photons emitted from sites of luciferase–luciferin interaction are detected using a cooled charge-coupled device camera and light signal intensity is illustrated on a false color scale (blue = low, red = high).

These studies show that it is possible to achieve targeted, local gene delivery. In addition, analyses of the cytokine
profile in draining lymph nodes and of levels of systemic anti-CII (type II collagen) antibodies revealed that there was no influence on systemic immune responses, supporting the local nature of this therapeutic approach. Moreover, antigen specificity was found to be required for long-term retention of the T cells in inflamed joints and for therapeutic effect. These findings are in keeping with those of Setoguchi et al. [2], who showed the antigen-specificity-dependent amelioration of murine AIA by IL-10-expressing T cells, which accumulated primarily in the inflamed joints and did not affect the systemic immune response to antigen.

A simple method for targeted gene delivery is the local injection of either naked DNA or viral vectors into, for example, inflamed joints. Interestingly, however, studies by Robbins and co-workers [20, 21, 52] in various models of arthritis show a remarkable ‘contralateral effect’ after intra-articular injection of adenoviral vectors encoding anti-inflammatory cytokines and cytokine antagonists; that is, disease amelioration was observed not only in the injected but also in the non-injected contralateral joints. These authors suggest that modified activity of DCs may be a possible mechanism underlying this phenomenon, because the adoptive transfer of DCs isolated from adenovirus-treated mice confers disease suppression [8**].

These observations prompted Robbins and co-workers [8**] to investigate further the use of genetically modified DCs in the therapy of autoimmune disease. They demonstrated that intravenous injection of immature DCs infected with adenovirus encoding IL-4 into mice with established CIA resulted in almost complete suppression of disease, with no recurrence of disease for up to four weeks after treatment. A recent report from another group supports these findings. Intradermal, intravenous and intraperitoneal injection of bone-marrow-derived DCs retrovirally transduced to express IL-4 before disease onset reduced the incidence and severity of CIA [9**]. These studies suggest that DCs could prove to be powerful vehicles for effective, long-term gene therapy of autoimmune disease.

A principal future task for gene therapy research in autoimmunity will be to apply these abundant findings from animal models to human disease. As a first step into this direction, phase I clinical trials of gene therapy for rheumatoid arthritis have been started in the US, the Netherlands and Germany. The American trial [53] was completed recently and showed safe and successful gene expression after ex vivo retroviral transduction and intra-articular injection of autologous synovial cells [53, 54].

Conclusions
Gene therapy as a novel means of treating autoimmune diseases has been extensively studied over the past few years. Various genes encoding anti-inflammatory cytokines, receptor antagonists and molecules that modulate apoptosis and signaling pathways have been administered by different routes and by viral and non-viral vectors, and have proved to be effective in preventing the development of disease and treating active autoimmune diseases.

As mentioned above, the first phase I clinical trial of gene therapy was recently completed in patients with rheumatoid arthritis. Thus, the feasibility, effectiveness and, to a certain extent, safety of gene therapy have been established. In order to introduce meaningful gene therapy as a therapeutic option in clinical medicine that surpasses our current nonspecific immuno-suppressive treatments, future studies will have to focus on optimizing safety and effectiveness and on safeguarding against systemic side-effects.

As different vector systems and different delivery systems have different effects in different diseases, we do not expect there to be one universal ‘magic bullet’ that cures all. Rather, in a given clinical situation the optimal treatment will need to be tailored to the therapeutic goals by using different weapons from the gene therapy arsenal. Aims for future research should therefore include, first, optimizing the target specificity of gene therapy; second, ensuring both local delivery of therapeutic gene products to the sites of inflammation and regulated gene expression to minimize systemic adverse effects; third, studying sensible combination therapies to maximize therapeutic benefit; and fourth, establishing the ideal gene, vector and delivery system for a given disease entity or therapeutic goal.

Antigen-specific T cells and DCs hold some promise as effective gene delivery vehicles. Novel ‘engineered’ chemokine receptor bearing cells that can home to inflammatory lesions to deliver ‘regulatory proteins’ is another approach that might be studied. Finally, as some very encouraging results have been obtained in studies combining various gene products and/or gene delivery strategies, future research should look further into combination therapy, which, in conventional pharmacological therapy using small molecule drugs, is generally accepted to achieve potentiated therapeutic effectiveness and to reduce side-effects. Gene therapy continues to be a very promising area of research for the advancement of treatment options for autoimmune diseases.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


Using retrovirally transduced T cells expressing luciferase and novel bioluminescence imaging, the authors demonstrate the in vivo trafficking and homing behavior of these gene delivery vehicles in real time, illustrating how local gene therapy can be achieved by local delivery of gene products.


The authors use a novel way of apoptosis induction in pro-inflammatory cells. Gene therapy application of the widely expressed β-galactosidase-binding protein galectin-1 is shown to abrogate clinical and histopathological manifestations of arthritis and induce increased susceptibility to antigen-induced apoptosis in draining lymph node cells.


These authors demonstrate that transduced DCs can be used to effectively and durably treat established autoimmune arthritis – thus introducing the use of DCs as a promising means of gene delivery.


In analogy to the study by Kim et al. [16*], this study establishes effective disease prevention by using genetically engineered DCs.


11. Nomoglycemia in diabetic mice is achieved by gene therapy replacement of insulin. The authors show that non-pancreatic cells engineered to express a cleavable pro-insulin can release mature human insulin sufficient to treat autoimmune diabetes.


These authors take the treatment approach of Falqui et al. [16*] one step further by introducing a rat LKP gene promoter into their SIA-encoding adeno-associated virus vector, thus achieving regulated SIA expression in response to blood glucose levels. Both studies introduce a concept that could be developed to be an attractive alternative to the current insulin replacement therapy using subcutaneous injections.


This study demonstrates that bone erosion, cellular joint infiltration, blood vessel formation and chemokine levels are affected differentially by adenovirus-encoded IL-4 when administered before or after disease onset. These findings provide some insight into the issue of the variable therapeutic success that has been obtained in different studies, despite the use of the same therapeutic molecule, for example, IL-4.


This manuscript describes a novel bicistronic, IRES-containing retroviral vector construct that allows efficient and durable transduction of antigen-specific T cells serving as vehicles for targeted gene delivery.

