

Annals of the Rheumatic Diseases

Leaders

Gene therapy in autoimmune diseases

Collectively, autoimmune diseases constitute a major, unmet, clinical challenge. Although no single autoimmune disorder is highly prevalent, there are over 80 of them, and 20% of the population is affected; approximately 75% of patients are women. Because these diseases are generally incurable and difficult to manage, there is a pressing need for novel approaches to their treatment. For reasons detailed below, we have proposed that gene therapy merits investigation in this regard.¹

Why genes?

Traditional pharmacological approaches to treatment entail the synthesis of small, diffusible compounds given orally or by injection. These approaches have yet to provide ideal agents for use in autoimmune diseases. Recent research, however, has identified a number of proteins with the potential to improve treatment, but these are difficult to administer long term. Gene transfer provides the opportunity to deliver protein products, as well as therapeutic species of nucleic acids, such as antisense RNA, much more efficiently than traditional methods of drug therapy. Furthermore sustained, *in situ* production of the gene products would eliminate the need for frequent re-administration. In addition, gene delivery and subsequent expression has the potential to be highly localised, if needed. Indeed, gene therapy's greatest strength may be its ability to produce high, sustained concentrations of therapeutic macromolecules within a defined anatomical location. In the case of RA, for example, anti-arthritis proteins are already being delivered systemically to patients by subcutaneous or intravenous injection. There is, however, no practical method for transferring these molecules selectively to joints in a sustained fashion.

Gene therapy

In its broadest sense, gene therapy is the transfer to patients of a gene, or genes, for therapeutic purposes. With some exceptions, such as skeletal muscle and skin, naked DNA is not efficiently taken up and expressed by mammalian cells. For this reason it is necessary to use vectors as agents of gene transfer and gene expression. Most vectors presently in use for gene therapy are based upon viruses; retrovirus, adenovirus, adenoassociated virus and herpes simplex virus are most frequently used for this purpose.² The wild type viruses have been genetically manipulated to render them incapable of replicating or otherwise causing disorders, while retaining their infectivity. Individual viral

vectors have their idiosyncratic advantages and disadvantages.² As a group they are difficult and expensive to produce in large amounts, and there are lingering concerns about safety. Non-viral vectors, such as liposomes, overcome some of these issues, but remain much less efficient than vectors based on viruses.

Of particular concern to chronic conditions, such as autoimmune diseases, is the need for vectors that either give long term gene expression or lend themselves to frequent re-administration. Integrating viruses, such as retrovirus and adenoassociated virus, become established within the chromosomes of the host cells and provide the best prospects for long term gene expression. For non-integrating vectors such as adenovirus, which are only transiently expressed, it is probable that repeated administration will require the vectors to be rendered non-immunogenic. For most applications, therapeutic genes encoding native, human proteins are likely to be used in which case the gene products should not be antigenic. Viral coat proteins, however, are highly antigenic; thus repeated administration of a viral vector will require the patient to be immunosuppressed or tolerant to the virus. Making patients tolerant to potentially pathogenic agents may be unwise but immunosuppressive drugs, already used as part of the standard treatment of autoimmune diseases, could be helpful. Many non-viral vector systems have the advantage of being non-antigenic.

Regardless of the vector system that is used, gene transfer to the target tissues may be accomplished by *in vivo* or *ex vivo* strategies. In the former, vectors are injected directly into the body. In the latter, cells are removed, genetically altered outside the body, and then returned to the individual. *Ex vivo* delivery is clearly more tedious, expensive, and cumbersome, but it permits selection and scrutiny of the genetically altered cells before re-implantation. For many types of cells that divide readily *in vitro*, but rarely *in vivo*, it permits use of Moloney-based retroviral vectors, able only to infect replicating cells. For these sorts of reasons, *ex vivo* gene delivery has made the greatest initial progress and most human gene therapy trials use this strategy. Nevertheless, the development of improved vectors for *in vivo* gene delivery is an area of active research that should eventually provide the means to deliver genes by simple injection. Once this has been accomplished, *in vivo* delivery will clearly become the method of choice for most human applications.

Table 1 Gene products of potential use in the treatment of autoimmune diseases

Product	Effect
Soluble CD40, CTLA ₄ , Fas L	Inhibit lymphocyte activation, may induce tolerance
Antibody to CD4, CD5, CD7, CD52, etc	Induces apoptosis in lymphocytes and other cells expressing Fas
Antibody to TCR specifically present on autoreactive T cells	Eliminate various subsets of lymphocytes. Inhibits T cell activation
IL12	Eliminates autoreactive T cells
IL10, IL4	Inhibits Th-2 lymphocyte activity
IL1Ra, sIL1RI, sIL1RII	Inhibits Th-1 lymphocyte activity
sTNFR1, sTNFR2	Antagonise IL1
IL4, IL10, IL13	Antagonise TNF
Antibodies to IL1, IL6, TNF, etc	Anti-inflammatory
Ribozymes, antisense RNA, decoy RNA	Inhibit their cognate cytokine
	Modulate gene expression

Finally, there is the issue of whether to deliver genes locally to discrete areas of disease activity or systemically, where the genes and their products become more generally distributed. Local delivery produces less side effects and is suitable for autoimmune diseases, such as Grave's disease, which primarily affects a discrete anatomical location. Systemic delivery, in contrast, seems better suited to the treatment of disseminated autoimmune diseases like lupus. It is unclear whether local or systemic delivery, or some combination of the two, would be most applicable to RA, which, although primarily affecting joints, has important extra-articular components.

Which genes to use?

To cure an autoimmune disease, it is necessary to eliminate the immune system's response to the inciting autoantigen. In the absence of a cure, it may be possible to treat the disease very effectively by suppressing those components of the immune response that lead to the disorder. Candidate agents in the latter context include antigen presentation, T lymphocyte activation, and cytokine function. An important distinction between curing and treating diseases, as outlined here, concerns the required period of gene expression. A gene that selectively eliminates autoreactive cells might need to be expressed for only a short period of time. A gene that treats the disease, in contrast, might need to be expressed for the life of the patient.

At our present state of knowledge, the best chance of a permanent effect in autoimmune disease is to eliminate autoreactive cells or to induce selective tolerance to the inciting antigen. Selective elimination of certain sub-sets of lymphocytes can be achieved with antibodies that recognise specific cell surface markers. There is presently much interest in eliminating autoreactive T cells by transferring genes encoding Fas ligand.³ Preliminary evidence suggests that selective tolerance can be achieved by blocking interactions between co-stimulatory ligand pairs such as CD40/CD40L and B7/CD28.⁴ Soluble CTLA-4 has emerged as a particularly promising molecule in this regard.⁵ This strategy has the advantage of not necessarily requiring knowledge of the autoantigen.

In many cases, the pathophysiology of autoimmune disease is driven by an imbalance in the activities of Th-1 and Th-2 lymphocytes.⁶ Redressing this imbalance offers an alternative approach to therapy. There is of particular interest in the use of interleukin 12 (IL12), a product of Th-1 lymphocytes, to treat diseases such as lupus in which Th-2 activity is thought to be excessive, and in the use of IL10, a product of Th-2 lymphocytes, to treat diseases such as inflammatory bowel disease where Th-1 activity is thought to be excessive. IL10 has the additional advantage of being, like IL4 and IL13, an anti-inflammatory cytokine with the ability to antagonise the biological activities of cytokines such as IL1 and TNF α .⁷ These two pro-inflammatory cytokines can also be inhibited with specific

antagonists such as soluble receptors and IL1Ra.⁸ Table 1 lists the proteins with potential use in autoimmune diseases.

Therapeutic gene products need not be limited to secreted proteins. Intracellular processes in the transduced cells may be targeted with intracellular proteins and species of RNA, such as anti-sense RNA and ribozymes. There is interest in using these approaches to block the actions of certain transcription factors, particularly NF- κ B and AP-1.^{9,10} One limitation of targeting intracellular molecules is that ex vivo gene therapy is unlikely to be useful, and very high transduction efficiencies are probably required.

Pre-clinical findings

Data from pre-clinical studies in a variety of different animal models permit guarded optimism about the future of gene therapy in autoimmune diseases. Raz *et al*¹¹ have shown that the immune responsiveness of mice can be selectively modulated by the intramuscular injection of naked, plasmid DNA. Injection of DNA encoding IL2 increased immune function, while injection of DNA encoding TGF β was immunosuppressive. Using this approach, it was possible to alter disease activity in murine lupus¹² and experimental colitis in rats.¹³

Most attention, however, has been directed toward treatment of rheumatoid arthritis, the most common autoimmune disease.^{14,15} An anti-arthritis effect has been noted after transfer to synovium of genes encoding IL1Ra,¹⁶⁻¹⁸ IL1sR, and TNFsR¹⁹; injection of "decoy" oligonucleotides containing NF- κ B⁹ and AP-1¹⁰ recognition sequences has also proved effective. In certain cases, a pronounced anti-arthritis effect has been noted in other joints on the same animal.^{9,18,19} This effect is clearly important as it, to some degree, meets the concerns of those who consider local gene therapy to be unsuited to a polyarticular, systemic disease like rheumatoid arthritis. An alternative way to tackle these issues is to transfer genes to cells such as lymphocytes with the potential to home to affected joints. There is preliminary evidence that this approach has merit.²⁰ Ex vivo retroviral transduction of T lymphocytes with IL 4 cDNA also reduces disease activity in mice with experimental autoimmune encephalomyelitis, a model of multiple sclerosis.²¹ Potentially anti-arthritis genes have also been transferred to the haematopoietic stem cells of mice.^{22,23} This produces high, lifelong, circulating levels of the gene product. Expression of IL1Ra and TNFsR in this way reduces certain responses to endotoxin,²³ suggesting that these products are biologically active and able to inhibit acute inflammatory reactions.

Human trials

The first human gene therapy trial for an autoimmune disease started last year.²⁴ Nine patients with RA will be treated by ex vivo retroviral delivery of a cDNA encoding human IL1Ra. Because this is the first human use of gene therapy for a disease that is not considered lethal, the

overriding priority has been to establish the safety of gene transfer to diseased joints. Among the safety features of this protocol are: the use of a gene whose product, IL1Ra, has a highly favourable toxicological profile; the use of ex vivo delivery so that cells may be thoroughly tested before re-implantation; extensive screening for replication competent retrovirus and other adventitious agents; surgical removal of the genetically altered joints as part of joint replacement surgery one week after gene transfer.

Details of the human protocol are given elsewhere.²⁵ To abbreviate, participants need to be post-menopausal or women with rheumatoid arthritis who have had an ovariectomy whose surgical management requires replacement of the metacarpophalangeal joints 2–5 and at least one other prior arthroplasty. Autologous synovium is recovered at the time of the prior joint surgery. Synovial fibroblasts are grown from it, and half the cultures are transduced with the retrovirus MFG-IRAP. Having confirmed that transduced cultures are secreting sufficient IL1Ra, aliquots of both the transduced and untransduced cells are extensively tested for a variety of adventitious agents and endotoxin. After satisfactory test results, the remaining cells are prepared for injection into the metacarpophalangeal joints. In a blinded fashion, two joints receive untransduced, control cells and the other two receive transduced cells secreting IL1Ra. One week later, synovia are harvested and tested for evidence of transgene expression and a local biological response to the transgene product. So far, five patients have been treated in this way. A similar trial was recently started at the University of Düsseldorf.

Conclusions

Gene therapy offers promising new avenues for treating autoimmune diseases. Very encouraging pre-clinical data have led to the first human trials. This permits optimism about the future development and clinical application of this approach to a wide spectrum of autoimmune conditions. Now that proof of principle has been established, the next goals are to determine the best gene, or combination of genes, to use in each disease, to develop improved gene delivery systems, and to achieve long term, regulated gene expression.

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- Evans CH, Robbins PD. Prospects for treating autoimmune and inflammatory diseases by gene therapy. *Gene Ther* 1996;3:187–9.
- Robbins PD, ed. *Gene therapy protocols*. Totowa, NJ: Human Press, 1996.
- Lau HT, Yu M, Fontana A, Stoeckert CJ. Prevention of islet allograft rejection with engineered myoblasts expressing FasL in mice. *Science* 1996;270:109–12.
- Parker DC, Greiner DL, Phillips NE, Appel MC, Steele AW, Duric FH, et al. Survival of mouse pancreatic islet allografts in recipients treated with allogeneic small lymphocytes and antibody to CD40 ligand. *Proc Natl Acad Sci USA* 1995;92:560–4.
- Finck BK, Linsley PS, Wofsy D. Treatment of murine lupus with CTLA41g. *Science* 1994;265:1225–7.
- Mosmann TR, Coffman RL. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Ann Rev Immunol* 1989;7:145–73.
- DeWaal-Malefyt R, Abrams J, Bennett B, Fidgor CG, DeVries JF. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 1991;174:1209–20.
- Moreland LW, Heck LW, Koopman WJ. Biologic agents for treating rheumatoid arthritis. *Concepts and progress. Arthritis Rheum* 1997;40:397–409.
- Makarov SS, Baldwin AS. Local suppression of NF-kB inhibits experimental arthritis in rats. *Arthritis Rheum* 1996;39 (suppl):S121.
- Shiozawa S, Shimizu K, Tanaka K, Hiro K. Studies on the contribution of c-fos/AP-1 to arthritic joint destruction. *J Clin Invest* 1997;99:1210–16.
- Raz E, Watanabe A, Baird SM, Eisenberg RA, Parr TG, Lotz M, et al. Systemic immunological effects of cytokine genes injected into skeletal muscle. *Proc Natl Acad Sci USA* 1993;90:4523–7.
- Raz E, Dudler J, Lotz M, Baird SM, Berry CC, Eisenberg RA, et al. Modulation of disease activity in murine systemic lupus erythematosus by cytokine gene delivery. *Lupus* 1995;4:286–92.
- Giladi E, Raz E, Karmeli F, Okon E, Rachmilewitz D. Transforming growth factor-beta gene therapy ameliorates experimental colitis in rats. *Eur J Gastroenterol Hepatol* 1995;7:341–7.
- Kang R, Ghivizzani SC, Herndon JH, Robbins PD, Evans CH. Gene therapy for arthritis: principles and clinical practice. *Biochem Soc Trans* 1997;25:533–7.
- Evans CH, Robbins PD. Pathways to gene therapy in rheumatoid arthritis. *Curr Opin Rheumatol* 1996;8:230–4.
- Otani K, Nita I, Macaulay W, Georgescu HI, Robbins PD, Evans CH. Suppression of antigen-induced arthritis by gene therapy. *J Immunol* 1996;156:3558–62.
- Makarov SS, Olsen JC, Johnston WN, Anderle SK, Brown RR, Baldwin AS, et al. Suppression of experimental arthritis by gene transfer of interleukin-1 receptor antagonist cDNA. *Proc Natl Acad Sci USA* 1996;93:402–6.
- Bakker AC, Joosten LAB, Arntz OJ, Helsen MMA, Bendele AM, Van De Loo FAJ, et al. Prevention of murine collagen-induced arthritis in the knee and ipsilateral paw by local expression of human interleukin-1 receptor antagonist protein in the knee. *Arthritis Rheum* 1997;40:893–900.
- Ghivizzani S, Kang R, Hatton C, Tio C, Lechman E, Emmert A, et al. In vivo delivery of genes encoding soluble receptors for IL-1 and TNF- α results in a synergistic therapeutic effect in antigen-induced arthritis in the rabbit knee. *Arthritis Rheum* 1996;39 (suppl):S308.
- Chernajovsky Y, Adama G, Podhajcer O, Müller G, Robbins PD, Felmann M. Inhibition of transfer of collagen induced arthritis into SCID mice by gene transfer of spleen cells with retroviruses expressing soluble human tumor necrosis factor receptor. *Gene Ther* 1995;2:731–5.
- Shaw MK, Lorens JB, Dhawan A, DalCanto R, Tse HY, Tran AB, et al. Local delivery of interleukin 4 by retrovirus-transduced T lymphocytes ameliorates experimental autoimmune encephalomyelitis. *J Exp Med* 1997;185:1711–14.
- Boggs SS, Patrene KD, Mueller GM, Evans CH, Doughty LA, Robbins PD. Prolonged systemic expression of human IL-1 receptor antagonist (hIL-1ra) in mice reconstituted with hematopoietic cells transduced with a retrovirus carrying the hIL-1ra cDNA. *Gene Ther* 1995;2:632–8.
- Doughty LA, Patrene KD, Evans CH, Boggs SS, Robbins PD. Constitutive systemic expression of IL-1Ra or soluble TNF receptor by genetically modified hematopoietic cells suppresses LPS induction of IL-6 and IL-10. *Gene Ther* 1997;4:252–7.
- McCarthy M. Gene therapy for rheumatoid arthritis starts clinical trials. *Lancet* 1996;348:323.
- Evans CH, Robbins PD, Ghivizzani SD, Herndon JH, Kang R, Bahnson AB, et al. Clinical trial to assess the safety, feasibility, and efficacy of transferring a potentially anti-arthritic cytokine gene to human joints with rheumatoid arthritis. *Hum Gene Ther* 1996;7:1261–80.