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Gene Therapy in Inflammatory Diseases

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Preface

Inflammatory diseases cast a persistent shadow over the wellbeing of mankind. Their effects range from the acute and reversible symptoms that accompany, for example, transient microbial infection to the progressive misery resulting from chronic conditions such as rheumatoid arthritis. Despite a sustained, huge effort by the pharmaceutical industry for much of the present century, these diseases remain difficult to treat and almost impossible to cure.

Inflammation is a biological process; it seems axiomatic that any logical attempt to improve its medical management requires a thorough understanding of this biology. Modern molecular approaches to the study of inflammatory diseases have provided an appropriate conceptual framework, and we are beginning to see the first fruits of this in the clinical application of biological agents as anti-inflammatories. Gene therapy is the latest modality to emerge from this trend.

The genetic components of most inflammatory diseases are complex, polygenic and quantitatively less important than environmental influences. Why, then, use gene therapy to treat them? The answer is that genes are not, at this stage, being used to compensate for genetic alterations or polymorphisms in the patient. They are, instead, being used to deliver therapeutic gene products or to alter levels of gene expression in the host. In this sense, genes are being used as biological delivery systems for the RNA and protein molecules they encode, or as molecular regulators of gene expression.

Gene transfer systems are necessary because traditional methods of drug delivery fail to deliver proteins and nucleic acids in a clinically useful fashion, especially in chronic inflammatory diseases where therapeutic concentrations of these molecules need to be maintained for extended periods of time. Moreover, parenteral delivery of such agents exposes unaffected organs to concentrations of the drugs that are no less than those seen by the affected organs. This leads to undesirable side-effects, a problem for which anti-inflammatory drugs are notorious. Gene transfer technology, in contrast, can achieve the sustained, targeted, local production of therapeutic gene products. In theory, the level of production can be regulated as required by the level of disease activity.

Although recent, these concepts have found wide acceptance and a growing number of investigators are now involved in research in this area. Completion of the first human clinical trial of gene therapy for an inflammatory disease provides a timely juncture for the publication of this volume, the first to be dedicated to inflammation gene therapy. The focus of this book is on chronic inflammatory diseases, as this is the area where gene therapy is likely to have its greatest impact – biological treatment of acute inflammatory conditions such as septic shock is more likely to involve the direct infusion of gene products than the genes themselves.

Rather than ask every investigator in the field to contribute a short chapter and thereby risk producing a series of disjointed, redundant mini-reviews of each author's own research, we have selected a senior figure from each of the major disease areas to write an authoritative review. Most major inflammatory disorders including arthritis, diabetes, asthma, salivary duct conditions such as Sjögren's syndrome, lupus erythematosus, multiple sclerosis, and organ transplantation, are covered in detail. Additional disorders, such as colitis and keratitis, are mentioned. The chapter on organ transplantation provides an interesting example of how an iatrogenic problem illuminates areas of naturally occurring disease. Physicians involved in the first clinical trials of gene therapy in inflammation have contributed a chapter describing these landmark studies.

We have deliberately avoided chapters on vectors and other technological issues, as these have been extensively described in many other books on gene therapy. Instead, such issues are discussed within the introductory chapter that reviews briefly the basic concepts of gene therapy in inflammatory diseases. Nevertheless, we thought it appropriate to include two chapters on the use of naked DNA, as this may have a special niche in certain anti-inflammatory settings. One of these is in DNA vaccination to eliminate autoreactive T lymphocytes and the other to achieve immune deviation.

Finally, there is a chapter describing the use of gene transfer to establish models of inflammatory diseases which are more informative and authentic than those resulting from standard genetic or non-genetic manipulations. These models should improve both the understanding and treatment of inflammatory conditions, and they serve to remind us that the preclinical development of gene transfer and gene therapy is not merely an exercise in technology but also a novel scientific means of investigating wide areas of disease pathophysiology.

There is every expectation that genetic treatments will enhance the anti-inflammatory armamentarium early in the next millennium. The present volume describes the state-of-the-art as we stand on the threshold of this quantum leap.

Pittsburgh, Pennsylvania, July 1999

Christopher H. Evans
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Gene therapy for inflammatory diseases – basic concepts

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Introduction

Although inflammatory diseases vary widely in the organs they affect and the symptoms they produce, as well as in their chronicity, morbidity and mortality, they share common pathophysiological pathways [1]. These pathways are illustrated in Figure 1.

The earliest changes occur in the vasculature where, in response to injury, infection or other initiating stimuli, the capillaries become leaky and the expression of adhesion molecules on their endothelial lining is increased. The latter leads to the capture of leukocytes which leave the circulation and enter the affected tissues by a process of diapadesis. The early infiltrate contains predominantly polymorphonuclear leukocytes which are replaced by mononuclear cells as inflammation progresses.

Within the inflamed tissue, both the infiltrating leukocytes and cells that are normally resident there secrete mediators, particularly cytokines and prostanoids, which perpetuate and magnify the inflammatory process in complex autocrine and paracrine fashions. These also provoke the production of proteinases, free radicals and other agents which destroy the integrity of the affected tissues.

This chain of events can be interrupted in a variety of ways. Traditional anti-inflammatory drugs include steroids, which suppress inflammation at multiple sites in the sequence shown in Figure 1, and cyclooxygenase inhibitors, which reduce the formation of prostanoids. Newer approaches target the adhesion molecules responsible for leukocyte recruitment and the cytokines that drive the disease locally. If the inflammation has an immune basis, there are various strategies for suppressing the immune response, particularly those which interfere with stimulation and co-stimulation of T lymphocytes by antigen-presenting cells (discussed in [2]).

Based upon the scheme shown in Figure 1, a number of gene products come to mind as potential anti-inflammatory agents (Tab. 1), including antibodies, soluble

Gene Therapy in Inflammatory Diseases, edited by C. H. Evans and P. D. Robbins

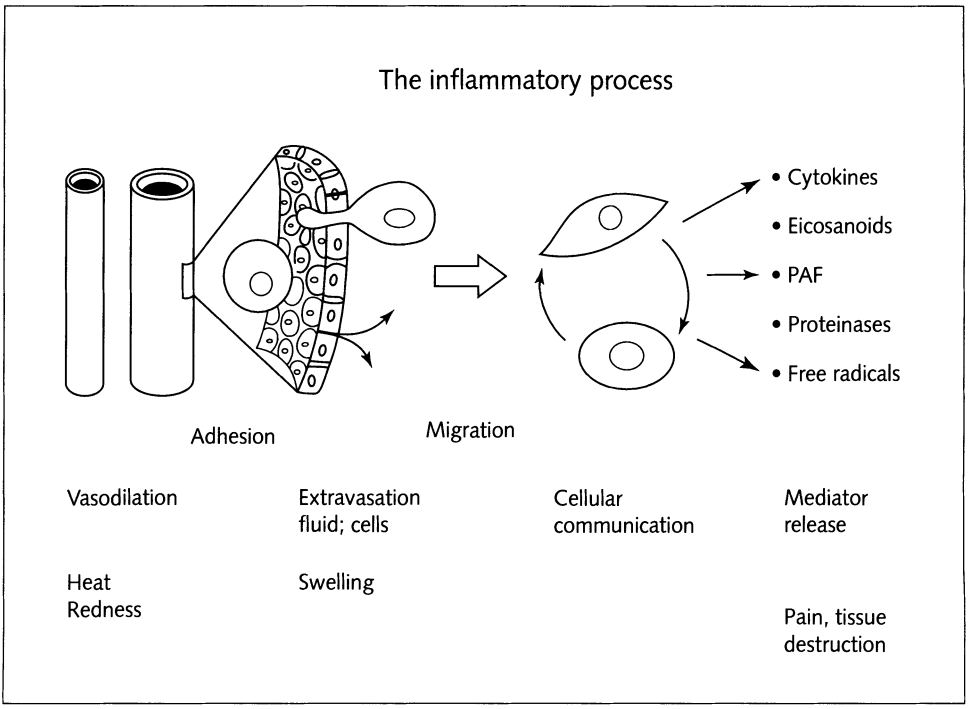

Figure 1
The inflammatory process. Taken from [1] with permission.

receptors or counterligands, type 2 cytokines or naturally occurring inhibitors such as the interleukin-1 receptor antagonist. These molecules have obvious potential application to the biological therapy of inflammatory diseases [3], but they are all proteins and proteins are poor drugs, especially in chronic conditions. The major problem is that of delivery.

Proteins are rarely effective when taken orally, as they are acidified and digested in the GI. They can be administered by various routes of injection, but this is unpleasant and impractical in chronic conditions. Moreover, because it is rarely possible to introduce the proteins in question directly and selectively into the site of disease, there is exposure of non-target organs and the prospect of deleterious side-effects. A particular concern with anti-inflammatory agents in this regard is the potential for increasing the incidence of infection and malignancy.

Gene therapy was proposed as a biological delivery system with which to circumvent these problems [2]. The original concept was to transfer genes encoding the therapeutic proteins of interest into sites of disease [4]. The affected tissues would then express the genes locally, thus producing the therapeutic gene products endoge-

Table 1 - Possible anti-inflammatory biological agents

Type of molecule	Examples
Anti-adhesion	Antibodies to, for example, ICAM-1 etc.; soluble forms of, for example, VLA4, CD44, etc.
Cytokine antagonist	Soluble receptors; IL-1 receptor antagonist
Anti-inflammatory cytokine	IL-4, IL-10, IL-13 and other Th-2 cytokines
Immunosuppressive	Soluble forms of T cell co-stimulatory molecules, e.g. sCTLA4; anti-TCR vaccines; antibodies to lymphocyte surface antigens; Th-1, Th-2 cytokines
Regulator of T cell differentiation	Type 1 cytokines (e.g. IL-12) in Th-2 driven disease Type 2 cytokines (e.g. IL-10) in Th-1 driven disease
Antioxidant	Superoxide dismutase, catalase, etc.
Anti-erosive	TIMPs, PAIs and other proteinase inhibitors
Regulators of gene expression	Antisense RNA, ribozymes, decoy nucleic acids, transcription factors, etc.

TIMP, tissue inhibitor of metalloproteinases; PAI, plasminogen activator inhibitor; ICAM, intercellular adhesion molecule; VLA, very late antigen

nously. In this way, systemic side-effects could be avoided and frequently repeated application would not be necessary. Moreover, there is the potential for regulating the level of gene expression to reflect disease activity.

Building upon these concepts, it is possible to envisage the delivery of not only proteins but also therapeutic species of RNA, such as ribozymes and anti-sense RNA [2]. Furthermore, the range of molecular targets has been expanded to include, for example, transcription factors (see Makarov, this volume) and certain death ligands (see Zhang et al., this volume) which induce apoptosis of inflammatory cells. DNA may also be used for the purpose of vaccinating against selected cell populations (see Godillot et al., this volume), and decoy oligonucleotides can act as regulators of gene expression (see Makarov, this volume).

Gene transfer vectors

Central to the success of any gene therapy protocol is an efficient means of transferring genes to the target cells in a way that gives appropriate levels of gene expression. Gene delivery vectors are employed to facilitate this process [5].

The simplest vector comprises a plasmid which contains the gene of interest under the control of a suitable promoter. Plasmids are inefficient gene delivery vehicles, but they hold much promise for DNA vaccination (see Godillot et al., this volume). For reasons that are not completely understood the introduction of plasmids into muscle and skin provokes strong, cell mediated immunity to the protein expressed from the plasmid. This property may be of use in vaccinating against autoreactive lymphocytes in autoimmune inflammatory diseases. There are also conditions where the low levels of gene expression that follow delivery of plasmid DNA are sufficient for an anti-inflammatory effect. As noted by Miyata et al., this volume, such effects have been reported for animal models of colitis, lupus, keratitis and arthritis.

The uptake and expression of plasmid DNA can be enhanced by associating it with a carrier. Liposomes, various proteins and certain polymers may be used for this purpose. Although these are usually more effective than naked DNA, the highest levels of gene transfer are achieved using viral vectors. These vectors exploit the natural ability of viruses to deliver their genomes to the cells they infect with great efficiency. For use as vectors, the wild-type viruses are genetically manipulated with the aim of eliminating their ability to replicate and cause disease, while retaining their infectivity. So far, four different classes of virus have been modified in this fashion with a success that permits their use in human clinical trials: retrovirus, adenovirus, adeno-associated virus and herpes simplex virus (Tab. 2).

Retroviruses have been used in the majority of human gene therapy trials, largely because of the ease with which they can be manipulated and manufactured, and because the cells they transduce express no viral proteins. Most of these vectors are derived from the Moloney murine leukemia virus and have as a major drawback their inability to transduce non-dividing cells. This has limited their use to *ex vivo* gene therapy where cells are removed from the body, infected with the retrovirus *in vitro*, and then re-implanted. The possibility of administering retroviruses *in vivo* by direct delivery has resulted from the development of recombinant retroviruses with titers as high as 10^{10} particles/ml. When the target cells are triggered to divide in situ, the *in vivo* delivery of high titer retroviruses can lead to efficient transduction [6]. Newer retroviral vectors derived from lentiviruses, which infect non-dividing cells, offer an alternative approach to the use of retroviruses for *in vivo* gene delivery [7].

Because retroviruses integrate their genomes into the chromosomal DNA of the cells they infect, they offer particular advantages in settings requiring long-term persistence of transgenes. Integration is, however, random and there is thus a finite, albeit small, possibility of insertional mutagenesis. There is thus much interest in utilizing adenoassociated virus (AAV) which, in its wild-type state, integrates into the host cell at a specific site at the tip of chromosome 19. Recent advances in the production of high titer, recombinant AAV have led to increasing experi-

Table 2 - Properties of present vectors

Vector	Advantages	Disadvantages
Integrating viral		
Retrovirus		
MoMLV-based	Straightforward production	Require target cell division
	No viral proteins made	Possible insertional mutagenesis
	Extensive use in human trials	
Lentivirus-based	Transduce non-dividing cells	More development required
AAV	Site-specific integration[1]	Difficult to produce
	Non-pathogenic	Small packaging capacity (4 kb)
	Transduce non-dividing cells	
	No viral proteins made	
Viral non-integrating		
Adenovirus	Straightforward production	Inflammatory
	High titers	Immunogenicity of transduced
	Transduce non-dividing cells	cells
HSV	Large packaging capacity	Difficult to produce
	High titers	Cytotoxic
	Transduce non-dividing cells	
Non-viral		
Naked DNA	Simple	Few cells transfect well
	Non-immunogenic	
	Inexpensive	
	Safe	
Liposomes	As above	Gene expression usually transient and low
Particle bombardment (gene gun)	Used in conjunction with plasmid DNA	Cumbersome; requires specialized equipment
DNA-ligand complexes	May be targetable	Possible antigenicity
	Receptor-mediated uptake often efficient	Low expression

[1] *Wild-type AAV integrates in a site-specific fashion. Recombinant virus appears as if it does not. Note that all types of vectors are the subject of considerable research. This table summarizes the present state of development.*

MoMLV, moloney murine leukemia virus; AAV, Adeno-associated virus; HSV, Herpes simplex virus.

Reproduced from [2] with permission.

mental use of this vector. In several instances impressive persistence of transgene expression has been reported [8]. It is unclear, however, whether recombinant AAV retains the ability to integrate into mammalian genomic DNA and, if so, whether this occurs in a site-specific manner. Disadvantages of AAV include its small packaging capacity of 4 kb, and its high antigenicity which serves as a barrier to repeated dosing.

Probably the most powerful vectors are derived from adenovirus which can be grown to very high titers, is highly infectious towards a wide range of dividing and non-dividing cells and accommodates up to 14 kb of packaging. Extremely high levels of gene expression can be attained using viral promoters such as the cytomegalovirus early promoter. Expression, however, tends to be temporary. This is partly explained by the non-integrating nature of adenoviral DNA. Additional reasons for transient expression are not fully understood but appear to have an immunological basis. The earliest adenoviral vectors had deletions engineered into the E1 and E3 regions of the viral genome. Cells transduced with these viruses continue to express viral proteins. Because these proteins are antigenic they trigger powerful immune responses, a problem exacerbated by the fact that the E3 region encodes an immunosuppressive protein. In response to this, second generation viruses deleted in the E1 and E4 regions have been made, but it is not clear whether the duration of transgene expression is increased by this means. Recombinant adenoviruses, known as "gutted" viruses, lacking all coding sequences have been generated and their performance remains under evaluation [9]. In addition to triggering immune responses [10], infection with the first generation adenoviral vector, alters the metabolism of the host cell by, for instance, activating MAP kinases [11] and NFκB [12].

Herpes simplex virus (HSV) forms the basis of an alternative non-integrating high titer, highly infectious vector [13]. HSV has a large genome which may accept 30 kb or more of packaging. Early generations of HSV vectors were cytotoxic but considerable engineering of the viral genome is beginning to produce vectors with improved properties. Because HSV establishes a natural latency in neurons, vectors derived from HSV have the potential to achieve prolonged transgene expression after infection of nervous tissue [14].

Because gene delivery remains the single most important obstacle to human gene therapy, considerable effort is being devoted to the improvement of these and other vectors. As well as addressing issues of the level and duration of transgene expression, vectors are being engineered to improve targeting, safety, stability and ease of production.

Gene therapy strategies

Genes may be introduced into the patient by direct, *in vivo* delivery or indirect *ex vivo* delivery (Fig. 2). The choice of strategy is determined *inter alia*, by the anato-

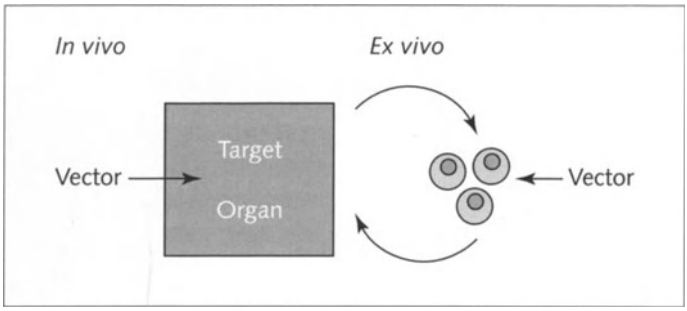

Figure 2
Models of gene delivery
In vivo *gene delivery involves the direct injection of genes into the body with the expectation that they will reach the target site and become incorporated into the target cell.* Ex vivo *gene delivery is a process whereby cells are removed from the body, are genetically altered* in vitro*, and are reimplanted in such a way as to reach the target organ. Taken from [20] with permission.*

my and physiology of the target organ and the vector that is used. For example, as discussed in the previous section, because Moloney-based retroviruses require division of the host cell for successful transduction, they are nearly always used in *ex vivo* protocols. Adenoviral vectors, in contrast, are most frequently used for *in vivo* gene delivery because they transduce non-dividing cells very effectively. Nevertheless, if it is difficult to gain access to the cells of choice *in situ* it may still be necessary to adopt *ex vivo* strategies regardless of the vector used. Examples of such cells include chondrocytes, stem cells and blood cells.

It is possible to draw a distinction between local and systemic gene delivery (Fig. 3). Local delivery implies that the genes are delivered to anatomically restricted sites where the gene product will accumulate, with minimal exposure of other organs. This has the advantage of minimizing side-effects while maximizing the local therapeutic effect. Local gene therapy is, however, poorly equipped to deal with disseminated disease. For this it may be necessary to utilize so-called systemic delivery where the secreted gene products gain access to the systemic circulation. This may be achieved by transferring genes to sites such as the liver, muscle and skin as well as by utilizing artificial organoids implanted into the host. Intravenous or intraperitoneal injection of adenovirus is one way to obtain high circulating levels of secreted transgene products.

It has recently become apparent that local gene delivery in models of inflammatory joint disease can bring impressive therapeutic effects at locations removed from the site of application (see Makarov, this volume). This appears to reflect the ability of inflammatory cells to traffick [15] and thereby blur the local/systemic distinc-

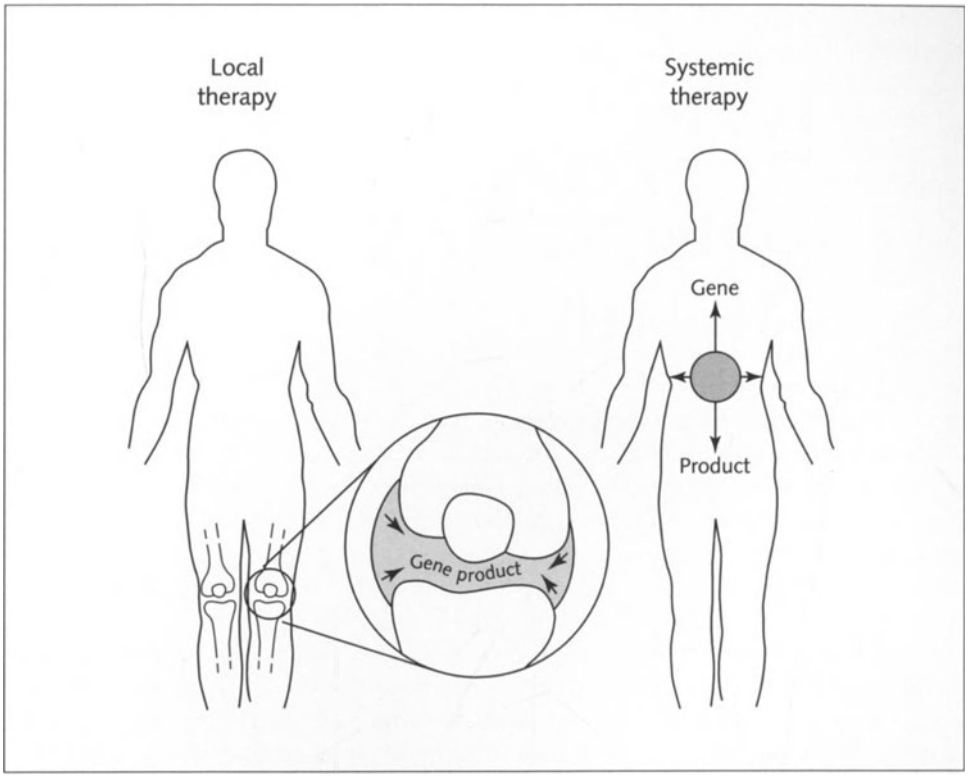

Figure 3
Local and systemic gene delivery
In local gene delivery, genes are delivered to individual sites of disease, such as the knee joint shown in this example. In systemic gene delivery, genes are delivered to sites where a secreted gene product has access to the systemic circulation. Taken from [20] with permission.

tion. Cells with the ability to home selectively to sites of inflammatory disease offer many advantages as vehicles for therapeutic gene delivery.

Regulation of gene expression

Although the expression of genes is regulated at several levels, in the context of gene therapy, attention has focused mainly on transcriptional regulation. Most investigators continue to use viral promoters that are either, as with the retroviral long terminal repeat, part of the original virus from which the vector was derived or, as with