Supplement Review

High-efficiency gene transfer into nontransformed cells: utility for studying gene regulation and analysis of potential therapeutic targets
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Chapter summary

The elucidation of the signalling pathways involved in inflammatory diseases, such as rheumatoid arthritis, could provide long sought after targets for therapeutic intervention. Gene regulation is complex and varies depending on the cell type, as well as the signal eliciting gene activation. However, cells from certain lineages, such as macrophages, are specialised to degrade exogenous material and consequently do not easily transfect. Methods for high-efficiency gene transfer into primary cells of various lineages and disease states are desirable, as they remove the uncertainties associated with using transformed cell lines. Significant research has been undertaken into the development of nonviral and viral vectors for basic research, and as vehicles for gene therapy. We briefly review the current methods of gene delivery and the difficulties associated with each system. Adenoviruses have been used extensively to examine the role of various cytokines and signal transduction molecules in the pathogenesis of rheumatoid arthritis. This review will focus on the involvement of different signalling molecules in the production of tumour necrosis factor alpha by macrophages and in rheumatoid synovium. While the NF-κB pathway has proven to be a major mediator of tumour necrosis factor alpha production, it is not exclusive and work evaluating the involvement of other pathways is ongoing.

Keywords: adenovirus, gene transfer, macrophage, NF-κB, rheumatoid arthritis

Introduction

The revolution in biological knowledge in the past two decades, with the ability to manipulate, sequence, clone and transfact DNA, has resulted in the sequencing of multiple prokaryotic and eukaryotic genomes, and has spawned a whole new industry, the biotechnology industry – now a major segment of the pharmaceutical industry. This has led to the development of important new drugs, such as cytokines, as well as monoclonal antibodies and receptor Fc fusion proteins.

In this chapter, we will present evidence, using tumour necrosis factor (TNF)-α as our example, that gene regulation is complex and varies depending on the cell type, as
well as the signal eliciting gene activation. Analysis of gene regulation is typically performed in transformed cell lines with, for example, footprinting analysis and DNase protection to evaluate which portions of DNA are ‘protected’ by transcription factors. With genes induced by multiple signals, such as TNF-α, however, it is not clear whether every signal operates in the same way in every cell. Prior literature suggested they did not, since in human T-cell lines (JURKAT), TNF-α synthesis was nuclear factors of activated T cells (NFAT)-dependent and NF-κB-independent, whereas in murine macrophages it was NF-κB dependent [1]. Hence, while a useful indicator, knowledge of the sites in the promoter for transcription factors would not have yielded any definitive data pertaining to a given circumstance.

We will also present evidence that there are new approaches of high-efficiency gene delivery that do permit the elucidation of gene function in physiological and pathological circumstances. The technique we have developed employs replication-deficient adenoviruses, but other efficient delivery systems can also be equally valuable for defining gene function. However, their utility may depend on the particular cell system being investigated.

The problem of gene delivery
Certain molecular techniques, while yielding new information, have limitations. DNA transfer by transfection has low efficiency in easily transfectable transformed cell lines (e.g. HEK 293, COS, CHO), but almost no efficiency in normal ‘primary’ cells. Low-efficiency DNA transfection need not be a rate-limiting step, as cells stably transfected with DNA can be detected, sorted or cloned. However, there are problems that cannot be overcome. One problem is that transfected cells from certain lineages such as macrophages, a cell type of major importance in inflammation and chronic rheumatoid diseases, are specialised to degrade exogenous material (such as DNA) and consequently do not transfect. Gene function in one cell type may not be identical to that in another; hence, conclusions made in transfectable cell lineages may not extrapolate to other nontransfectable cells. Furthermore, transformed cell lines have enormous abnormalities in their genomes, with deletions, duplications and alterations in chromosome number.

We therefore explored methods of DNA delivery to nontransformed cells, both normal and cells from pathological sites, that yield effective DNA transfer into >90% of cells. This level of transfer would permit the inhibition of signalling pathways by the inserted DNA. This may encode an endogenous inhibitor (e.g. inhibitor of kappa B alpha [IκBα], which binds NF-κB), a dominant negative (i.e. blocks normal or ‘wild type’ product), mutation of a kinase or transcription factor, a DNA encoding an antisense mRNA, or even a DNA encoding a single-chain antibody variable region.

Methods of DNA delivery
Current gene delivery systems can be broadly categorised into two main groups: nonviral (plasmid DNA, DNA-coated gold particles, liposomes and polymer DNA complexes), and viral vectors (adenovirus, retrovirus, adeno-associated virus, lentivirus and herpes virus).

Nonviral vectors
Nonviral vectors attract considerable interest as they lack some of the risks inherent with viral vectors. Nonviral DNA delivery is less toxic, less immunogenic, and easier to prepare. However, DNA delivery is significantly less efficient and gene expression only occurs over a short duration, posing serious limitations and opportunities for the use of these systems for long-term treatment of rheumatic and other diseases. In addition, inflammatory responses following in vivo delivery of DNA and lipid–DNA complexes have been reported [2–4]. The exact mechanism is currently unknown, but might be related to the presence of unmethylated CpG motifs in plasmid DNA propagated in bacteria.

Naked or plasmid DNA
DNA can be directly administered to skeletal muscle via an intramuscular injection of ‘naked’ plasmid DNA. However, gene expression was restricted to cells nearest the route of injection, and less than 1% of the plasmid DNA was incorporated into the cells [5]. Systemic administration has been even less successful. Intravenous injection of naked plasmid DNA showed no gene expression, even in the liver, where the highest uptake was observed [6]. Liu et al. have more recently shown that it is possible to achieve systemic administration of naked DNA under hydrostatic pressure by injecting a large volume of DNA solution via the tail vein of mice [7]. Although these experiments demonstrate that it may be possible to deliver naked DNA to cells, this is not a feasible option for exploring gene function.

Gene gun
Particle-mediated (‘gene gun’) gene transfer has been applied to various cells and tissues. Gold particles coated with plasmid DNA are propelled at the target tissue at pressures of 150–200 psi. The efficiency of this system varies, with skin cells showing the greatest uptake of 10–20% [8]. This system has been used to deliver a nucleic acid-based hepatitis B vaccine to both mice and humans, and is presently in clinical trials [9]. The major limitations are the shallow penetration of particles, associated cell damage, and the inability to deliver the DNA systemically.

Cationic lipids and polymers
Cationic lipids, such as cationic derivatives of cholesterol and diacylglycerol, quaternary ammonium detergents, and lipid derivatives of polyamines condense plasmid DNA to form particles based on the electrostatic interaction and to
poly(lysine) have been widely used as carrier backbones. Poly(amino acids) available, poly(glutamic acid) and at condensing DNA than cationic lipids. Among various modified. These cationic polymers are also more effective to stimulate an inflammatory response.

Cationic polymers (amino acids) are soluble in water, are biodegradable, are not significantly immunogenic, and have multiple functional groups that can be chemically modified. These cationic polymers are also more effective at condensing DNA than cationic lipids. Among various poly(amino acids) available, poly(glutamic acid) and poly(lysine) have been widely used as carrier backbones.

Viral vectors
Since the evolution and survival of viruses is based on their ability to infect cells and to replicate their genes, it is not surprising that viral vectors have proven to be the most efficient vehicles for DNA delivery to cells both in vitro and in vivo. Viruses also permit greater duration of transgene expression. This is particularly relevant when attempting to treat chronic conditions, such as rheumatoid arthritis (RA), osteoarthritis, cystic fibrosis, or muscular dystrophy, where enduring transgene expression is desirable. Viral vectors are capable of delivering exogenous cDNA to the synovium, enabling effective levels of intra-articular transgene expression following direct injection to the joint. The expression of certain gene products has proven to be sufficient to inhibit the progression of disease in animals with experimental arthritis, even when the virus is administered after onset of inflammation (see later).

Adeno-associated virus
Adeno-associated virus (AAV) is a single-stranded DNA parvovirus that causes no known pathology in humans. AAV infection involves attachment to a variety of cell surface receptors (heparan sulfate, integrins, and fibroblast growth factor receptor 1) followed by clathrin-dependent or clathrin-independent internalisation [11]. Although the wild-type AAV integrates into a specific locus (AAVS1) on the human chromosome 19, recombinant AAV vectors do not because they lack the rep gene. Consequently, gene expression is transient [12]. Transgenes of up to 5 kb can be accommodated within the 4.68 kb single-stranded DNA virus, and AAV infects a wide variety of dividing and nondividing cells. Recombinant AAV encodes no viral proteins, reducing its immunogenicity and capacity to stimulate an inflammatory response.

Two different groups have recently shown that AAV encoding IL-4 promotes long-term (up to 7 months) protection from articular cartilage destruction and amelioration of disease severity in mice with collagen-induced arthritis (CIA) [13,14]. Additionally, intra-articular injection of AAV encoding soluble TNF receptor I significantly decreased synovial hyperplasia, and cartilage and bone destruction, in TNF-α transgenic mice [15].

Herpes simplex viruses
The herpes simplex virus (HSV) offers several potential benefits that could be useful for treating arthritic conditions. More than one-half of the 152 kb genome of HSV is not required for replication in vitro, and large genes or multiple genes can therefore be accommodated by this vector. HSV also develops latency in certain cell types, where the viral genome persists for the life of the host cell without integrating into the genome and without altering host cell metabolism. Although most of the immediate-early genes are inactivated, HSV vectors still express low levels of some viral proteins, resulting in an inflammatory response [16]. Administration of HSV-derived vectors as DNA–liposome complexes improves in vivo transduction efficiency by evading the host anti-HSV immunity during systemic administration [15,17]. The delivery of a HSV vector encoding human interleukin-1 receptor antagonist (IL-1Ra) to the joints of rabbits with experimental arthritis has been shown to ameliorate inflammation [18].

Retroviruses
Retroviruses derived from the Moloney murine leukaemia virus have been used extensively in the laboratory and in clinical trials. They are able to incorporate 8–10 kb exogenous DNA, and they contain no native viral coding sequences. Retroviruses integrate their genome into that of the host cell, resulting in sustained transgene expression. This feature of retroviruses also presents a potential hazard because insertional mutagenesis could occur following random integration of the provirus into the host genome. This vector is currently used to treat the rare lethal immune disorder, severe combined immunodeficiency-X1, in children [19]. Successful application of these vectors for the study of gene function and gene therapy, however, has often proven difficult because they are unable to transduce quiescent cells [20].

Immortalised embryonic DBA/1 fibroblasts, infected with a retrovirus expressing murine IFN-β ex vivo and subsequently implanted intraperitoneally into mice immunised with bovine type II collagen, were able to prevent the onset of arthritis or to ameliorate existing disease [21]. Recent work by Croxford et al. has shown that fibroblasts, transduced with retroviral vectors expressing IL-10, could inhibit experimental allergic encephalomyelitis, which is a central nervous system autoimmune disease mediated by the action of CD4+ T cells, macrophages, and proinflammatory cytokines [22].
Transgene expression, similar to that observed with \textit{ex vivo} administration, has been achieved using direct administration of high-titre retroviruses into the arthritic joint [23,24]. However, high-titre preparations of Moloney murine leukaemia virus-based vectors have been difficult to generate routinely, and this has limited studies involving direct retroviral-mediated gene delivery.

The only clinical trial on gene therapy of RA involving nine patients was performed in the USA using a retroviral vector containing the cDNA for IL-1Ra. Synovial tissue was removed by joint surgery, and cells were expanded and infected \textit{in vitro} before being re-introduced into the joint space. Synovial tissue was removed 1 week later by joint arthroplasty for analysis, showing evidence of IL-1Ra expression at both mRNA and protein levels. This study of local \textit{ex vivo} retroviral gene therapy of RA demonstrated that safe and effective transgene expression could be achieved [25].

\textbf{Adenoviruses}

The remainder of this review will focus on the development and use of adenoviruses \textit{in vitro} and \textit{in vivo}. Replication-deficient adenoviruses are one of the most widely used systems for preclinical experimentation owing to their efficiency and technically straightforward methods for generating high-titre recombinant adenoviruses [26,27]. Adenoviral particles are \textasciitilde{}70–100 nm non-enveloped icosahedrons that contain a \textasciitilde{}35 kb double-stranded DNA genome and can infect a wide range of cell types.

Adenoviruses are endocytosed by attachment of their fibre protein to the coxsackie virus and adenovirus receptor (CAR) [28]. This process is facilitated by interactions between the penton base protein and integrins on the cell surface. This latter interaction induces release of the fibre protein and initiates internalisation of bound virions into early endosomes via clathrin-coated pits. After internalisation and endosomal escape, adenovirus particles undergo a stepwise disassembly programme and, finally, delivery of viral DNA to the nucleus. This viral DNA exists as extra-chromosomal DNA, resulting in transient gene expression (Fig. 1). The first generation of adenoviruses commonly used for preclinical research are devoid of the E1, E3 regions of the viral genome (Fig. 2). This prevents virus replication while providing the space required to insert your gene of interest. The ability of adenoviruses to infect nearly 100\% of cells, quiescent and dividing, makes it an extremely useful vehicle for gene delivery.

\textbf{Heterogeneity of gene regulation: dependent on cell type and signals}

Adenoviruses have been used to analyse macrophages (monocytes differentiated for 2 days with macrophage colony stimulating factor) stimulated to produce TNF-\(\alpha\) by a range of signals. Lipopolysaccharide (LPS), UV light, and phorbol 12-myristate 13-acetate (PMA) were shown to be NF-\(\kappa\)B dependent, while zymosan and anti-CD45 were NF-\(\kappa\)B independent, demonstrating that within a single cell type the stimulus determined the signalling pathway [29]. Again there were differences in T-cell-
dependent stimulation of macrophage TNF-α production, with T-cell receptor-activated T cells showing NF-κB-independent TNF-α production whereas bystander cytokine-activated T cells were driving NF-κB-dependent TNF-α [30]. The production of TNF-α in antigen-activated T cells was different from that in macrophages because in cell lines it appeared to be NF-κB independent.

A variety of cytokines and cytokine inhibitors produced by macrophages have been tested for their dependence on NF-κB using the IκBα adenovirus. It appears that NF-κB regulates essentially all proinflammatory cytokines, although not with every stimulus. Second, NF-κB does not regulate anti-inflammatory cytokines, such as IL-10, IL-11 or IL-1Ra, but has moderate effects on shedding of soluble TNF receptor. Third, there were differences within a single study (e.g. LPS) in the degree to which different proinflammatory cytokines were NF-κB dependent. IL-6 was ~80% inhibited, whereas TNF and IL-1 were 60% inhibited and IL-8 was only inhibited by 30–40%. The results varied with different stimuli; for example, with TNF-α stimulation, IL-8 is >60% NF-κB dependent. The reasons for these differences are not known. However, it is possible that other transcription factors are used for IL-1 and IL-8 production in response to LPS [31].

Work by Pope et al. has shown that PMA-treated macrophages, infected with a dominant negative CCAAT/enhancer binding protein beta (C/EBPβ) adenovirus, produce 60% less TNF-α in response to LPS. Furthermore, dominant negative versions of both C/EBPβ and c-Jun, but not NF-κB p65, suppressed PMA-induced TNF-α secretion, demonstrating that C/EBPβ and c-Jun contribute to TNF-α regulation in normal macrophages [32].

The importance of using the appropriate model for studying gene regulation, and thereby identify potential targets for therapy, has been aptly demonstrated by studies on the NF-κB-inducing kinase (NIK). This enzyme was identified as kinase for IκK1/α, a part of the IκB kinase (IKK) signalosome. Several studies on NIK have shown evidence that NIK acts as a gateway for multiple activators of NF-κB, including the key inflammatory factors, TNF-α, IL-1 and LPS. However, these studies have been exclusively performed in transformed cell lines [33–35]. We confirmed these results in cell lines using an adenovirus encoding a dominant negative version of NIK. However, introduction of the same construct into primary human cells of fibroblast, myeloid or endothelial lineage showed no role for NIK in TNF-α, LPS or IL-1 signalling (Figs 3 and 4) [36]. Rather, the role of NIK was restricted to NF-κB induction by activation of the lymphotoxin beta receptor (LTβR) (Fig. 4) [36]. This supported studies in cells from mice defective in NIK expression or function that showed that NIK function was restricted to LTβR signalling [37]. Together, these data highlight the diversity inherent in signalling pathways and the potent effect that transformation has on cell function.

Use of adenoviruses for analysis of RA and its models

In vitro analysis on synovial tissue

As the pathology of RA is concentrated in the synovium, analysis of this tissue can provide major insights into its pathogenesis. A key question was therefore whether adenoviruses could effectively infect human synovial cells in vitro and, if so, whether all cell types infect equally. It was found that >90% of synovial cells were infected at a multiplicity of virus of 40:1 using a β-galactosidase
Furthermore, it was established by flow cytometry that all subpopulations of cells were infected; macrophages, fibroblasts and also >80% of T cells. The surprising result indicated that cells from pathological sites are easier to infect than resting cells, probably due to their prior exposure to cytokines. This observation enabled us, in parallel with others, to evaluate the role of various signalling molecules in rheumatoid synovium.

With the IκBα adenovirus, we showed that multiple pro-inflammatory cytokines are downregulated: TNF-α by 70%, IL-6 by 80%, and IL-1 by ~50% (as shown in Fig. 5). In contrast, anti-inflammatory mediators were not affected. These results parallel prior studies with macrophages. The variable degree of inhibition indicates that, while NF-κB is of major importance, other important transcription factors are involved, especially for IL-1 and IL-8. The blockage of NF-κB resulted in the downregulation of degradative enzymes, with matrix metalloproteinases MMP-1 and MMP-3 markedly reduced. In contrast, the tissue inhibitor of matrix metalloproteinases, TIMP-1, was not downregulated [38]. Taken together, the local effects of NF-κB blockade would be very beneficial for RA. Much work has been carried out to define the inducers of NF-κB and to subsequently generate inhibitors (e.g. inhibitors of IKK2).

Adenoviral-mediated gene delivery of a non-degradable IκBα, or dominant negative versions of C/EBPβ or c-Jun, has been examined to determine the contribution of each transcription factor to IL-6 and IL-8 expression by RA fibroblast-like synoviocytes. Inhibition of NF-κB activation significantly reduced the spontaneous and IL-1β-induced secretion of IL-6 and IL-8. Conversely, inhibition of C/EBPβ and c-Jun/AP-1 had little or no effect on the production of either IL-6 or IL-8 [39].

The p38 mitogen-activated protein kinase (MAPK) is an important regulator of cytokine production, including TNF-α. While drugs blocking p38 MAPK (e.g. SB203570) were effective at blocking LPS-induced TNF-α, they were less effective at blocking spontaneous synovial TNF-α production. Specific p38 MAPK inhibitors, encoded in adenoviral constructs, were constructed to examine their effectiveness in rheumatoid synovial cultures. It was found that up to 80% of spontaneously produced TNF-α was inhibited (Ciesielski et al., manuscript in preparation).

**In vivo effects on animal models of arthritis**

Adenoviral vectors have been extensively used in preclinical studies on gene therapy of RA using a number of animal models of arthritis. Two main strategies have been employed: systemic delivery, whereby vectors are injected intravenously; and local delivery, whereby genes are transferred to the joint, either via direct injection into the joint (*in vivo*), or by infecting autologous synovial cells *in vitro* and transferring the transformed cells into the joint (*ex vivo*).
Modulation and suppression of experimental arthritis has been achieved by the systemic gene delivery of a variety of anti-inflammatory and immunosuppressive proteins. Sustained amelioration of murine CIA was demonstrated in our studies. An adenovirus encoding IL-10, when injected intravenously, produced elevated serum levels of murine IL-10 that reduced cell-mediated immune reactivity and IFN-γ production without affecting antibody responses. Interestingly, IL-10 was also able to suppress adenovirus-induced hepatic inflammation, a limitation of the first-generation adenoviral vectors [40].

Systemic delivery of an adenovirus encoding the cytotoxic T lymphocyte antigen CTLA4-Ig, an inhibitor of the CD28/CD80 and CD86 interaction between T cells and antigen-presenting cells, has also been investigated by our group. This treatment was similarly very effective, even if administered after onset of arthritis, being just as effective as protein delivery in optimal concentrations [41]. With the systemic injection of an adenovirus encoding an immunogenic protein, strikingly different results were obtained. The human p55 TNF receptor coupled to a mouse IgG Fc fusion protein gene, inserted into an adenoviral vector and...
injected systemically in arthritic mice, induced elevated serum levels of the fusion protein and ameliorated CIA for up to 7–10 days. Then, a dramatic inflammatory rebound was observed and treated mice appeared sicker than controls. These findings confirm prior reports on the role of host immune reactions elicited more towards the product of gene transduction than to the virus itself.

The group of Robbins and Evans has reported successful treatment of murine CIA with an adenovirus encoding IL-4 [42]. This treatment was also effective in the rat model of adjuvant arthritis after disease onset, and decreased levels of TNF-α, IL-1β, macrophage inflammatory protein-2, and CCL5 (the chemokine ligand for CCR5) were reported [43].

A local intra-articular approach has also been investigated using adenoviral vectors. As mutations in the tumour suppressor protein p53 are observed in synovial cells from some RA patients, overexpression of p53 by adenoviral gene transfer in synovial cell cultures in vitro, and in synovial tissue in vivo, has been achieved in a rabbit model of arthritis, and resulted in significant apoptosis. In addition, the intra-articular injection of p53 adenovirus determined a rapid induction of synovial apoptosis in the rabbit knee joint without affecting cartilage metabolism [44].

Makorov et al. have shown in a rat model of arthritis that local injection of a retrovirus expressing a mutant, non-degradable IκBα, induces improvements in joint disease [45]. This work is supported by experiments using another member of the NF-κB pathway; inhibitor of NF-κB kinase beta (IKKβ). Intra-articular injection of adenovirus encoding IKKβ-wt into the joints of normal rats caused significant paw swelling and histologic evidence of synovial inflammation. Increased IKK activity was detectable in the IKKβ-wt-injected ankle joints, coincident with enhanced NF-κB DNA binding activity, while a dominant negative version of IKKβ significantly ameliorated disease severity.

Figure 5

(a) Efficiency of infection of rheumatoid arthritis (RA) joint cell cultures. RA joint tissues were obtained from the synovium from patients with RA undergoing surgery. Cells were infected with Advβgal ( moi 40) for 48 hours prior to analysis of the cells for βgal expression by fluorescence activated cell sorting (FACS). (B) Effect of IκBα transgene expression on cytokine expression in RA joint cell cultures. RA joint cells were infected with AdvIκBα or AdvO ( moi 40). Culture supernatants were harvested after 48 hours and analysed for the expression of various cytokines by enzyme-linked immunosorbent assay. Data are representative of samples from at least five patients. IL-1RA, interleukin-1 receptor antagonist; TNF, tumour necrosis factor; sTNFR, soluble TNF receptor.
of adjuvant arthritis, and determined a significant decrease in NF-κB DNA expression [46].

The work described here regarding animal models of arthritis has been supplemented by a number of studies using retroviruses that were used to infect cell lines in vitro and then injected systemically in vivo [47].

**Future prospects**

The working draft covering 97% of the human genome was completed in June 2000. It is expected that the ‘finished’ sequence of greater than 99.99% accuracy will be completed within the next year. Analysis of the current sequence shows 38,000 predicted genes and many of these have no known function. Expectations within the pharmaceutical industry have been high that this knowledge will be transformed into therapeutic targets and new drugs. These expectations have so far not been met because crucial roadblocks remain in the elucidation of target genes for drug development. Expectations have been raised about the possibility of performing drug development ‘in silico’ using genetic information, but successes from this approach remain to be achieved.

The ability to explore gene function both in vitro and in vivo is paramount to the elucidation of gene regulation and potential targets for therapeutic intervention. While substantial work has been undertaken to determine the signalling pathways controlling TNF-α production and other inflammatory mediators, the roles of a multitude of cytokines, cytokine receptors, kinases and phosphatases remain to be explored.

Research into developing high-efficiency vectors for transgene delivery is a continually evolving field of research. Current research into developing cationic lipids/polymers for cell-specific delivery via receptor-mediated endocytosis, such as galactose, mannose, lactose, transferrin, epidermal growth factor, asialoglycoprotein and antibodies, may provide valuable advances allowing the targeting of specific cell populations [10]. Combining advances in DNA delivery via directed cationic lipids and polymers, and improved adenoviral or other viral vectors, looks to be a promising approach to evade clearance and to deliver stable gene expression to specific cells [48,49].

Although adenoviruses have proved valuable tools for preclinical research, the first generation of E1, E3 deleted vectors still contains native viral-coding sequences expressed at low levels, resulting in inflammatory responses (Fig. 2) [50]. Furthermore, expression of these viral proteins leads to the clearance of transduced cells, limiting transgene expression [51]. Recently described helper-dependent, or gutless, adenoviral vectors may overcome many of these problems. These adenoviruses do not encode any viral coding sequences and have shown an excellent expression profile in a variety of animal models, as well as reduced toxicity after local or systemic delivery [52]. Expression of the transgene can be detected for many months and the gutted adenoviruses do not elicit an immune response in animals previously immunised against the same adenovirus serotype [53].

**Concluding remarks**

There has been much progress in the development of methods of gene transfer into normal cells and cells from pathological sites. These techniques can be used to test the function of genes, allegedly known or unknown both in vivo and in vitro. These approaches can be used to elucidate important pathways, and to help confirm or define therapeutic targets.

**Glossary of terms**

AAV = adeno-associated virus; C/EBPβ = C/EBP enhancer binding protein beta; HSV = herpes simplex virus; IKK = IκB kinase; IKKβ = inhibitor of NF-κB kinase beta; IL-1Ra = interleukin-1 receptor antagonist; LTβR = lymphotxin beta-receptor; NIK = NF-κB-inducing kinase.

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