13

GENE THERAPY APPLICATIONS FOR THE TREATMENT OF ACUTE INFLAMMATORY CONDITIONS

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INTRODUCTION

Gene therapy represents a new therapeutic modality for the treatment of acute and chronic inflammatory diseases. Clinical trials are currently underway in patients with hereditary diseases, such as cystic fibrosis and α_1-antitrypsin deficiency (1) as well as in chronic infectious diseases, like hepatitis C and HIV infections, or in cancer (2,3). Although there has been a great deal of enthusiasm for gene therapy, it still remains unclear and too early to determine whether this new approach will be safe and effective.

Most approaches to gene therapy in humans have focused on curing or treating genetic diseases, either those arising through the germ line, or occurring in somatic tissues due to genetic injury or viral infections. In general, these approaches strive to permanently alter the genetic pattern of the host, and rely on gene transfer techniques that result in sustained or permanent expression.

Over the last decade, more than 300 phase I and II gene therapy clinical trials have been initiated for the treatment of inherited or acquired genetic disorders and cancer (4-6). There have been some well publicized early successes, especially in patients with adenosine deaminase deficiency (severe combined immunodeficiency syndrome) as well as some tragic adverse events, such as with the death of a patient with ornithine transcarbamylase deficiency receiving adenovirus gene therapy (7). Despite continuing improvements in the area of vector design, it is reasonable to conclude at

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present that both non-viral and viral based vectors will require further refinement before widespread application in patient populations comes about. The use of gene therapy is well-supported conceptually when the goal is to deliver genes to cells that lack the specific gene or where the gene is abnormal and malfunctioning. However, it is less well recognized that gene therapy techniques have much broader applications than merely the modification of endogenous gene expression. As recent studies have shown, non-genetic illnesses, such as acute and chronic inflammatory diseases (rheumatoid arthritis, delayed wound healing, acute inflammation) could also benefit from this approach (8-10).

We and others have argued that a less well-considered application for gene therapy is as a novel approach to deliver and target protein based therapies (9,11,12). Gene therapy is and can be an effective tool to target the expression of protein based therapies to individual tissues. By modifying the vector and the promoter system, a high level of tissue specificity and regulation of expression can be achieved.

ADVANTAGES OF GENE THERAPY

Compared to traditional drug delivery systems for protein-based therapies, gene therapy offers several theoretical advantages for the treatment of acute inflammation. The first is the ability to target specific tissues and to deliver genes effectively to the site of inflammation without systemic administration (Figure 1). This is accomplished by both physical means, as well as by using the precision of genetic specificity. Physical targeting of the vector can be achieved by different routes of administration, such as intranasal or aerosoled vectors (13,14) or by endoscopic administration. Although similar techniques can be used with protein based therapies, the larger size and specificity of the viral or nonviral vectors tend to restrict their distribution more effectively than protein-based therapies when administered locally. However, the power of targeting gene therapy to individual tissues rests primarily with the natural tropism of viral vectors and the specificity of tissue specific promoters. By taking advantage of the natural infectivity of many viruses for specific cells or tissues, or by using cell- or tissue-specific promoters, the specificity of the gene therapy for individual tissues can be dramatically increased. For example, following intravenous injection of either adenovirus or adeno-associated virus, viral expression of the transgene is restricted primarily to the liver (15). This route of administration offers a powerful advantage to treat liver diseases, such as liver cancer or hepatitis (16). Coupling this viral tropism with the use of a hepatocyte specific promoter, such as the albumin or phenylalanine hydroxylase promoter will assure that expression is limited exclusively to hepatocytes. Similar specificity can be achieved in neuronal

tissues with the neural specific enolase promoter in adeno-associated virus constructs (17). One of the most powerful approaches developed over the past couple of years has been to identify a tissue specific promoter that is upregulated in response to inflammation. This can be achieved in the liver using the endogenous promoters for hepatic acute phase reactant proteins, such as complement factor 3 (18). Thus, gene expression in the target tissue is activated only during the inflammatory response, and is theoretically shut off when the inflammation subsides. Perhaps one of the most attractive promoters which is inducible by inflammation is the pancreas-associated protein I promoter (19) whose expression in the pancreas is increased over 100-fold by pancreatic injury.

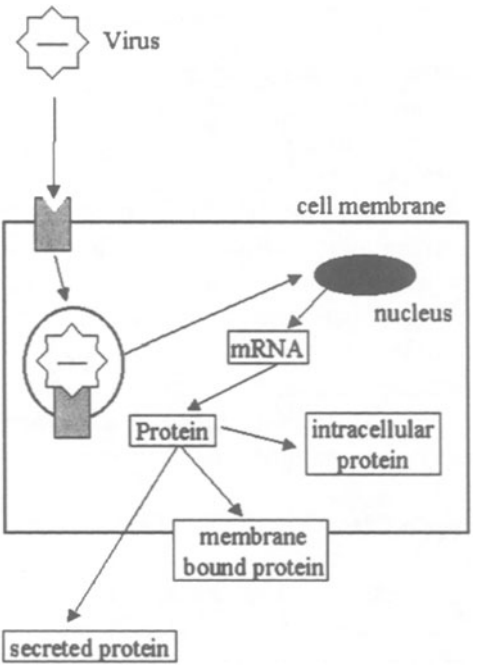

Figure 1. Viral infection of the host cell. Depending on the transgene, either intracellular, membrane bound or secretable protein is produced. Intracellular protein can enhance or inhibit signaling pathways. Membrane bound and extracellular protein take influence on other cells in the same compartment or even on more distant cells or proteins.

Recently, designer vectors have been conceived where a tissue specific promoter/enhancer is combined with an inducible or repressible promoter system, like the tetracycline-regulated or ecdysone inducible promoter. These

promoters offer an additional mechanism by which the expression of a particular gene can be regulated. Gene expression is dependent not only on the tissue specificity of one promoter, but also on the application of the drug which regulates expression of a trans-acting co-promoter or enhancer. This is very important when constant expression of a gene is not desired (20,21).

An additional theoretical advantage of gene therapy over protein based therapeutics is sustained expression. If the duration of therapy is to last for days or weeks, gene therapy has the advantage of sustained expression of the gene leading to fewer or even a single application, whereas conventional drug delivery is dependent on the pharmacokinetics and pharmacodynamics of the drug. Protein-based therapies generally have half-lives ranging from minutes to hours, whereas a single delivery of a gene lasts days to months depending upon the vector (22,23).

One of the most attractive advantages of gene therapy is the opportunity to regulate intracellular signaling pathways. The systemic or localized administration of protein based therapies are in general limited to compounds that act primarily through membrane-associated, receptor mediated events. Although endocytosis or pinocytosis of proteins occurs and provides an opportunity for intracellular trafficking of administered protein, the vast majority of internalized proteins are degraded in the endosome or lysosome. In contrast, gene therapy provides the opportunity to directly regulate the expression and activity of intracellular cell-signaling intermediates. The potential therapeutic opportunities are enormous, and early studies to date have examined the effects of overexpression of dominant negative IκB and Bcl-2 on hepatocyte injury, apoptosis and inflammation.

Choosing the Appropriate Vector for Gene Therapy in Inflammatory Diseases

There is no single gene therapy vector that is appropriate for all clinical uses (Table 1). Similarly, none of the current gene therapy approaches are without significant theoretical and practical limitations. The ideal vector for the treatment of acute inflammation will not necessarily be the same vector used for the treatment of chronic inflammatory diseases, cancer or genetic diseases. Rather, each disease entity and target protein will have different requirements for an optimal gene therapy approach. There are, however, several common characteristics of an ideal vector for use in acute inflammatory diseases. Expression of the gene should be rapid and transient, ideally limited to the duration of the inflammation. Peak expression should be reached within hours. The vector itself should not induce an additional immune reaction or exacerbate existing immune or inflammatory responses. Furthermore, the expression of the gene product should be limited to a

compartment with specificity to some cell types. In summary, the goal of gene transfer in acute inflammation is a rapid onset of expression (within hours), limited to the target compartment and subsiding within days or at the most weeks.

Table 1. **Non-viral and Viral Gene Transfer Vectors**

Non-viral vectors	Viral vectors
Plasmid DNA: Cationic Liposome	Adenovirus
Naked DNA (bacterial plasmid)	Retrovirus
Biolisitic Delivery (Gene gun)	Lentivirus
	Adeno-associated virus (AAV)
	Herpes simplex virus

NON-VIRAL MEDIATED GENE TRANSFER

Plasmid DNA:Cationic Liposomes

Cationic liposomes are positively charged lipid particles that bind with DNA by ionic interaction. Cationic liposomes range in size from 25-500 nm in size. Gene transfer takes place by fusion of the cell membrane with the lipid particle and the formation of an endosome. Plasmids are generally supercoiled circular DNA, usually of bacterial origin. Without specific surface receptors, liposomes are rapidly incorporated into a variety of cell types. This makes liposome-mediated gene transfer an attractive approach for cells and tissues that lack viral receptors. Despite the rapid uptake of plasmid DNA with cationic liposomes, transduction efficiencies are generally quite low, usually one or two logs than viral-based transduction schemes (Table 2). This is due in part to degradation of the plasmid DNA in the endosome and cytosol prior to nuclear entry and episomal transcription. In addition, transport of charged DNA through nuclear pores is very inefficient.

Although liposomes are generally considered to be relatively nontoxic and non-immunogenic, there is accumulating evidence that they elicit an inflammatory response (Table 2). More importantly, there is growing evidence that plasmid DNA, due to its bacterial origins, may elicit activation of the innate immune response (24). Earlier studies had suggested that both the systemic and local administration of liposome:DNA complexes were safe and well tolerated (4,10). However, in recent years concern has arisen that DNA in general, and bacterial DNA in particular, can elicit an innate immune response. Structural differences in bacterial DNA (CpG motifs) appear to be recognized by the host innate immune system as a signal of infection (5,6). Bacterial DNA and synthetic oligonucleotides derived from

bacterial DNA and grown in *E. coli* can be as efficient as endotoxin in activating macrophages and dendritic cells, and in stimulating the release of pro-inflammatory cytokines such as tumor necrosis factor α (TNFα) (25). Although the implications of these findings are presently unknown, the pro-inflammatory nature of plasmid DNA has raised concerns about its safety and utility, especially during acute inflammatory diseases.

Table 2. **Advantages and Disadvantages of Gene Transfer Vectors**

Vector	Advantages	Disadvantages
Non-viral vectors	not infectious	non specific
	no limit to the size of the DNA	low transfection rate
	low degree of toxicity	
Retrovirus	no known toxic effect	Random insertion of viral genome (possible mutagenesis)
		rapid degradation through complement
Lentivirus (HIV-1)	stable gene expression	serum conversion to HIV-1
Lentivirus (FIV)	stable gene expression	Limited insertion of gene
		lack of specific integration (possible mutagenesis)
Adenovirus-associated virus (AAV)	nonpathogenic and nontoxic	only small DNA can be inserted
Adenovirus	large DNA can be inserted	Inflammatory response by host
		Toxic

In two recent reports, we have shown that the timing of plasmid-based gene transfer can have a dramatic effect on the magnitude of the inflammatory response. In early 1995, we demonstrated that administration of a bacterial plasmid expressing human IL-10 complexed to cationic liposomes improved outcome and reduced the inflammatory response to lethal endotoxemia (26). Similar results were seen when an IL-10 expressing plasmid was administered prior to acute pancreatitis (27). However, when a similar plasmid containing a reporter gene was administered one day **after** the onset of acute pancreatitis, mortality was actually increased (28). Administration of the cationic liposomes and plasmid DNA increased TNFα mRNA expression in the inflamed pancreas and in the liver.

These results differ from the findings of Brigham and colleagues. They demonstrated in models of endotoxemia and acute lung injury that

application of plasmids complexed to cationic liposomes could be delivered intravenously as well as intratracheally and be expressed in vitro as well as in vivo (29,30) showing the feasibility of the use of plasmids for gene delivery. Others have shown that transfection efficiency of liposomes can be increased by incorporation of a cell-surface protein, such as integrin. It has also been demonstrated recently that such non-viral vectors transfect bronchial and alveolar cells nearly as efficiently as viral vectors (31).

Liposome-mediated CFTR (cystic fibrosis transmembrane conductance regulator) gene transfer in a clinical trial with cystic fibrosis patients showed a transient restoration of the deficit in some of the patients (32). In general, however, transduction efficiencies were low compared to viral approaches.

VIRAL MEDIATED GENE TRANSFER

Retro-and Lentivirus

Retroviral vectors are generally based on the Moloney murine leukemia virus (MMLV) and have a broad cell tropism. They are small enveloped RNA viruses that replicate through a DNA replicate. After internalization of the viral genome and reverse transcription into proviral DNA the viral genome is integrated into the host DNA. Retroviruses primarily infect dividing cells. They have been used in many gene therapy clinical trials for the treatment of cancer (6,33) and AIDS (34,35). Lentiviral vectors are based on human immunodeficiency virus (HIV)-1 or on feline immunodeficiency virus (FIV) (36,37). Neither of these viruses has been used in clinical trials and concerns still remain using HIV-1 based vectors because of the issue of serum conversion of the patients to HIV-1 (Table 2). Whereas, retroviruses can only infect dividing cells as they require the dissolution of the nuclear membrane to be able to deliver the preintegration complex into the nucleus (38), lentiviruses can infect nondividing cells as well (37). Depending on the therapeutic use one or the other may be more applicable. Infection of dividing cells is suitable for cancer, whereas it cannot be applied in other diseases, such as genetic disorders, where the target cell would be non-dividing cells such as hepatocytes or neurons (33,39).

Adeno-associated Virus

Adeno-associated viruses (AAV) are one of the smallest viruses, containing a linear, single-stranded DNA genome (4.7kb) (40). It is a nonpathogenic human parvovirus whose replication is facilitated by co-infection with a helper-adenovirus or herpes-virus for (41). Recombinant AAV produces long-term gene expression *in vivo* without immune response or toxicity (42) (Table 2). AAV transfects both replicating and non-replicating cells. Due to its small size and strict packaging requirements, however, only small

transgenes (<5kb) can be delivered. Development of hybrid viral capsid structures or the use of expression cassettes with small promoters will enable larger transgene incorporation (43,44).

Adenovirus

Adenoviruses are linear, double stranded DNA viruses surrounded by capsid proteins. By deleting specific regions of the viral genome, particularly the E1 region, and inserting a desired sequence under control of a constitutive viral promoter, the virus becomes a replication-incompetent vector capable of transferring the exogenous DNA to differentiated, non-proliferating cells (Figure 2). Adenovirus has a large insertional capacity for foreign genes, in the range of 7-8kb. By deleting more of the genome of the adenovirus (usually the E3 or E4 regions) even larger DNA fragments can be inserted. Adenovirus is highly efficient in transfecting epithelial cells; it is common to see greater than 50% of a target cell population transduced. Due to these properties, adenovirus-mediated gene therapy is very attractive for a variety of disorders, such as inflammatory diseases, cancer and neurologic disorders (Table 2).

However, there have been 2 intrinsic limitations to adenoviruses that hinder their widespread use as a gene therapy vector: dose limiting toxicity and cell-mediated and humoral immune responses. The immunological and inflammatory response of the host to the adenovirus varies depending on the dose of the virus, the site of delivery and the type of adenovirus, as well as the transgene being expressed (45). Intravenously administered adenovirus shows remarkable tropism for the liver, and primarily transfects hepatocytes (46). Although 90% of the virus is eliminated in the first 48 hours of infection by the innate immune response, as many as 95% of the hepatocytes are transfected after intravenous injection (47). Transgene expression generally lasts only 2-3 weeks with peak expression occurring during the first week (45). Loss of expression appears to be due in part to elimination of virally infected cells, primarily through apoptotic processes and a cytotoxic T-cell response. TNFα also appears to play a major role in the clearance of the virus (48). Treatment of animals with a TNF binding protein (bp) results in prolonged gene expression and reduces the inflammatory response (23). The duration of transgene expression is also limited because the adenoviral genome does not integrate into the host chromosomal DNA (33,49). Therefore, both immunogenicity and lack of adenoviral genome integration into the chromosomal DNA contribute to limited transgene expression.

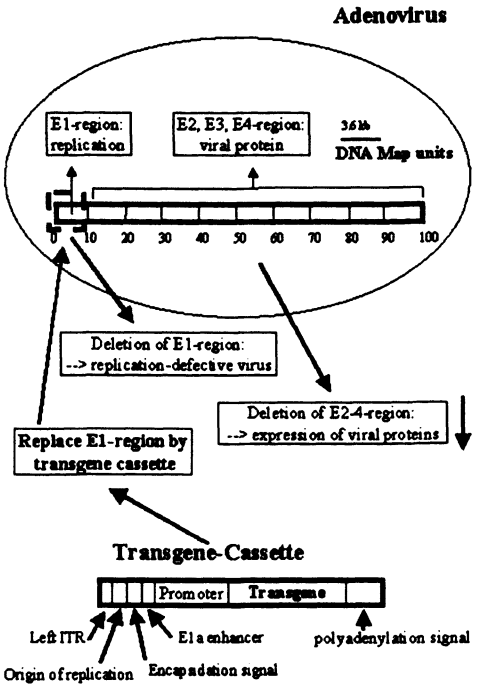

Figure 2. First-generation adenovirus construct. Replication deficient adenovirus constructs are generated by deletion of either E1 and/or E3 regions and replacement with a cassette containing the promoter, transgene, and associated sequences. These viral recombinants are replication deficient, but can be propagated in cell lines expressing E1 proteins. Deletion of the region determines the immunogenicity of the virus due to the transcribed viral proteins. The cassette containing the transgene can be inserted either in region E1 or E2-4.

Immunogenicity derives from expression of adenoviral early genes (E1, E2, E3 and E4) (33). Deletion of the E1 region (1st generation virus) is essential for generating replication-incompetent adenoviral vectors (Figure 2) (50). New approaches are being pursued to decrease the immunogenicity: deletion of E2 and E4 regions in order to avoid expression of viral proteins within the transfected cells (39). Investigators are attempting to generate vectors with no viral genes by constructing completely gutted vectors that contain only the viral terminal repeats and the packaging sequence (51,52). Deletion of not only the E1 region but also the E4 region of the adenovirus decreases expression of viral proteins, and reduces apoptosis of infected cells leading to a blunted immunoreaction (53).

GENE THERAPY AS A THERAPEUTIC MODALITY IN ACUTE SYSTEMIC INFLAMMATION

Acute inflammation, especially in critically ill patients, is associated with a Systemic Inflammatory Response Syndrome (SIRS), which is often followed

by a Compensatory Anti-inflammatory Response Syndrome (CARS) (Figure 3) (54). The former is characterized by exaggerated levels of pro-inflammatory cytokines, such as interleukin (IL) 1 and IL-6, whereas the latter is a state of immunosuppression and anergy, in which anti-inflammatory cytokines (IL-4, IL-10) predominate and immune cells are anergic. Septic shock is often associated with Multiple Organ Dysfunction Syndrome (MODS). Most patients survive the initial SIRS event and the pro-inflammatory state ultimately resolves. The prinflammatory cytokines and humoral mediators responsible for the induction of the innate immune response and SIRS also contribute to the development of acquired or specific immune defects. The role that apoptosis plays in sepsis syndromes has not been adequately explored to date, but there is rapidly developing evidence to suggest that increased apoptotic processes may play a determining role in outcome (55). In particular, increased apoptosis, particularly in lymphoid tissues and potentially in some parenchymal tissues from solid organs, may contribute to the sepsis-associated MODS, and can be a potential therapeutic target for intervention. Direct apoptotic organ injury, and the immune

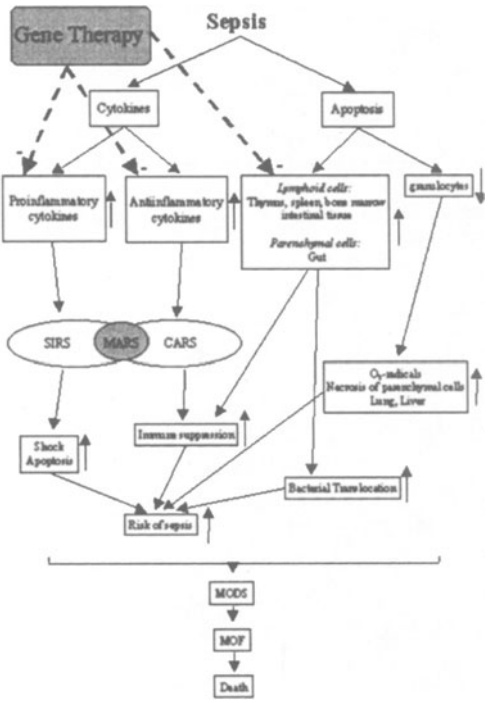

Figure 3. Sepsis induced cascades as a target for gene therapy. Gene therapy can be used to inhibit different pathways following onset of sepsis. Depending on the time point of intervention either the proinflammatory or antiinflammatory cytokines can be counterregulated or apoptosis can be inhibited.

suppression secondary to apoptotic losses in T-cell, B-cell and Natural Killer cell populations may contribute significantly to the risk of secondary opportunistic infections. Therapeutic efforts at modulating the apoptotic response, particularly by interfering with cell signaling pathways that lead to caspase-mediated apoptosis, represent an attractive therapeutic target for the septic patient. Identification of the cell signaling pathways that lead to and antagonize apoptosis has now become a major research focus.

During sepsis and SIRS, there are a number of potential therapeutic targets that would be amenable to gene therapy approaches. Ideally, the exaggerated synthesis of pro-inflammatory cytokines could be inhibited by interfering with signal transduction, for example by inhibition of nuclear factor (NF)-κB through overexpression of its inhibitor, Iκβ, or to suppress inflammation with anti-inflammatory cytokines (56) (Table 3). Blocking the activity of cytokines could be achieved by overexpression of TNF receptors (p55 or p75) or IL-1 receptor antagonist (IL-1ra), which have already been proven to be effective in endotoxin-induced lethality and adult respiratory distress syndrome (ARDS), rheumatoid arthritis and neuronal injury (26,57-60). These protein products frequently have short half-lives, which makes delivery through gene therapy very attractive. Furthermore, binding of these inhibitors competes with the natural ligand, so to be effective they need to be in considerable higher concentrations (100 to 1000 fold). This is often very difficult to achieve by protein delivery compared to gene therapy (61,62).

Several gene therapy approaches have been applied to the forced expression of either anti-inflammatory proteins or to cytokine inhibitors. Much of these studies have focused on efforts to reduce the magnitude of the pro-inflammatory cytokine response to an otherwise lethal endotoxin or bacterial challenge. In 1995, we demonstrated that pretreatment of mice with cationic liposomes and mammalian expression plasmids expressing IL-10 or the shed p55 TNF receptor were effective in reducing mortality and pro-inflammatory cytokine responses to a lethal endotoxin challenge (26) (Table 3). IL-10 based therapies appeared to be superior to the forced expression of p55, in part because the low concentrations of p55 obtained (10-100 pg/ml) appeared to be insufficient to neutralize all of the TNFα. In subsequent studies, Baumhofer and Rogy used a similar approach with the forced overexpression of IL-13 or IL-4 and showed similar efficacy (63). In those cases, they too could demonstrate improvements in outcome.

Denham and colleagues extended these findings to a model of acute pancreatitis (27). Mice were pretreated with a cationic liposome and expression plasmid delivering IL-10 and then subjected to a caerulin-induced model of fulminant pancreatitis. Pretreatment with the vector expressing IL-

10 resulted in significant improvements in outcome, as well as reductions in the pro-inflammatory cytokines, TNFα and IL-1. Surprisingly, however, post-treatment of mice with the same vector after the onset of pancreatitis actually worsened outcome. This latter result was presumed to be secondary to the exacerbation of the inflammatory response by the plasmid DNA.

One novel approach has been to block the early proximal inflammatory response by interfering with endotoxin signaling through its CD14/*tlr* complex. Wong and colleagues have recently reported that transfecting endotoxin sensitive mice with an adenoviral vector delivering the mutant *tlr4* receptor complex from C3H/HeJ mice conferred protection (64).

Gauldie and colleagues used adenoviral vectors expressing IL-10 to explore its utility in models of endotoxemic shock (65). Mice were subjected to intramuscular or intravenous injections of a first generation adenoviral vector expressing either IL-10 or a reporter vector. Mice treated with the adenoviral vectors expressing IL-10 also had improved outcome and reduced production of the pro-inflammatory cytokines. Drazan and colleagues performed a similar study in newborn mice. Intraperitoneal injection of an adenoviral vector expressing viral IL-10 transfected primarily the liver, and suppressed not only the plasma cytokine responses to a lethal endotoxin challenge but also reduced hepatic expression of TNFα and IL-1 (66).

Adenoviral vectors delivering the cytokine inhibitor, IL-1 receptor antagonist, have been shown to reduce mortality to endotoxemic shock, experimentally induced arthritis, autoimmune diabetes, and cerebral ischemic injury (67-69).

As previously mentioned, one of the most exciting aspects of gene therapy for acute inflammation has been the ability to directly modulate intracellular cell signaling pathways. This approach has been used most recently by Taylor and Geller who transfected hepatocytes with a dominant negative IκB, which effectively prevents NF-κB translocation and the activation of NF-κB dependent genes (70). Hepatocytes overexpressing this IκB repressor failed to induce nitric oxide synthesis in response to a cocktail of pro-inflammatory cytokines. These findings suggest that intracellular signaling pathways are amenable to regulation by these adenoviral constructs. Griesenbach and colleagues have recently transfected respiratory epithelium with adenoviral vectors expressing a dominant negative IκB, or with liposomes and NF-κB decoy oligonucleotides and have shown reduced IL-8 secretion, suggesting that this approach will be effective in reducing lung inflammation (71).

An alternative approach for gene therapy in bacteremia or sepsis syndromes has been to augment host anti-microbial properties (Table 3). Greenberger and colleagues noted in rats subjected to an intratracheal instillation of Klebsiella that IL-12 expression was markedly increased, and blocking IL-12 with antibodies resulted in a marked bacterial proliferation (72). When an adenoviral vector expressing both the p35 and p40 subunits of IL-12 were instilled into the lungs of rats, mortality from the lung Klebsiella infection was markedly reduced. Similarly, Abina and colleagues explored adenovirus induced over-expression of thrombopoietin on the prevention of septicemia and anemia secondary to myelosuppression in the mouse (73). These investigators noted that a single injection of an adenoviral vector expressing thrombopoietin prevented leukopenia and mortality from a myeloablative regimen.

During the later state of CARS (if it has not been prevented by early intervention) activation of immune cells with interferon (IFN)-γ or with IL-12 treatment could increase the immune response (74). Alternative approaches are overexpression of prostacyclins or free-radical scavengers to attenuate inflammatory changes. Both have been shown to be effective in animal models of endotoxin and hyperoxia-induced lung injury by non-viral and viral gene delivery (75,76).

Recently, emphasis has shifted from efforts to reduce the magnitude of the pro-inflammatory response to acute inflammation, to preventing the immune suppression that is frequently associated with increased apoptosis of lymphoid organs and intestinal epithelial cells in septic patients (55). This has also been seen in murine models of polymicrobial sepsis. Mice have increased apoptosis of lymphocytes in thymus and spleen (77,78), which can be reduced by overexpression of antiapoptotic proteins or treatment with caspase inhibitors (79,80). To inhibit apoptosis either antiapoptotic proteins (Bcl-2, Bcl-xL) can be overexpressed or upregulated, (for example by IL-10)(81), or pro-apoptotic proteins (Bax, Bid) can be inhibited (82,83). Further potential targets to inhibit apoptosis are apoptosis inhibiting factors (AIF) or even preventing activation of caspases-3 or —9 (Table 3). Overexpression of Bcl-2 has been shown to reduce hepatic injury by adenoviral vectors as well as hepatocyte death to FasL and TNFα. These findings suggest that apoptotic induced cell death will likely be amenable to gene therapy.

There are several daunting challenges that must be overcome before anti-apoptotic therapies can be considered in the septic patient. These are for the most part technical challenges aimed at successfully targeting both the appropriate signaling pathway and the specific cell population. In contrast to

increased lymphocyte apoptosis, delayed apoptotic removal of neutrophils has been imputed in the pathogenesis of adult respiratory distress syndrome (Figure 3) (56,84). Inhibition of neutrophil apoptosis through NF-κB dependent pathways in sepsis or SIRS appears to prolong the life of neutrophils once they have extravasated the blood compartment into lung parenchymal tissues, and to potentially increase oxidative damage in the lung. Thus, targeted blockade of apoptosis in lymphocyte populations must be specific enough to primarily target those lymphocyte cell populations undergoing increased apoptosis, and to be sufficiently transient to prevent the risk of malignant transformation associated with prolonged blockade of cell death.

Table 3. Therapeutic targets for gene therapy during sepsis depending on the time point of intervention.

Time point of intervention	Possible intervention
Early reaction to microbes	↑Anti-microbial properties (IL-12, thrombopoietin)
SIRS (proinflammatory cytokines dominate)	Signal transduction (↑IκB) ↑Antiinflammatory cytokines (IL-4, IL-10) ↑Receptors (TNF) or receptor antagonists (IL-1ra) Mutant receptors
CARS (antiinflammatory cytokines dominate)	Immune stimulation (↑IFNγ, IL-12)
Apoptosis (↑ or ↓ depending on cell type, i.e. lymphocytes, neutrophils)	Antiapoptotic proteins (Bcl-2, Bcl-xL, AIF, caspase inhibitors) Proapoptotic proteins (Bid, Bax)
Others	↑Prostacyclin, radical scavengers

CONCLUSIONS

During the past several years vector design has allowed for a broader range of therapeutic applications for gene transfer. Yet, the development of vectors for gene therapy is not sufficient enough as not all requirements have been fulfilled. Several issues need to be resolved and optimized before gene therapy can be applied routinely to patients.

Non-viral gene transfer seems very attractive because of its limited immunogenicity, transduction of a variety of different cell types, and transient expression, but transduction efficiency will need to be improved before it becomes a viable technique. In contrast, adenoviruses offer many advantages of non-viral vectors, but at present are limited by dose-dependent inflammation as well as development of an immune response, which

precludes repeated injections. As recent studies have shown, however, further modification of the viral genome may decrease the immune response to the virus. Clearly, further progress in the development of appropriate vectors will make gene therapy more attractive for treating acute inflammatory diseases.

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