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RATIONALE FOR THE USE OF GENE THERAPY FOR CRITICAL ILLNESS

Kenneth L. Brigham, M.D. and Roberto Cruz-Gervis, M.D.
Vanderbilt University, Nashville, TN 37221 USA

INTRODUCTION

Management of critically ill patients includes many challenges for physicians dedicated to their care. Treating these patients is complex, not only because of the acute and continuously changing manifestations of severe illnesses, but also because of the various complications that arise from invasive monitoring and therapy. Much progress has been made since the advent of Intensive Care Units (ICUs), mainly through improved supportive care. Even though current ICU care has translated into a reduction in ICU mortality, not much ground has been gained in the management of the specific disease processes that contribute to ICU mortality. Among these disease processes, acute lung injury (ALI) and its more severe form, the acute respiratory distress syndrome (ARDS), septic shock and multiple organ dysfunction syndrome (MODS) are clearly the most common contributors to unfavorable outcomes. Despite the institution of standard therapy aimed at the underlying process, many patients die secondary to the deleterious effects of the exaggerated systemic inflammatory response and end-organ damage seen in these disease states.

Current management strategies have been unsuccessful in treating or halting the progression of the more severe critical illnesses (i.e., septic shock, ARDS, MODS) leading to the unacceptably high mortality rates currently seen for these conditions in the ICU. For these reasons it is imperative that novel therapies be developed, which can be effective in halting or reversing the biological and clinical events that characterize critical illnesses. Among the novel therapies being currently considered gene-based therapy is one of

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the most promising modalities. By safely and efficiently delivering genes that code for inflammatory inhibitors or anti-inflammatory mediators, gene therapy may be a potent means of blocking the progression of the systemic inflammatory cascade.

PATHOGENESIS OF ACUTE LUNG INJURY (ALI) AND MEDIATORS OF THE SYSTEMIC INFLAMMATORY CASCADE

A significant body of knowledge has been gained in regards to the pathobiology of sepsis syndrome and ARDS, and many inflammatory mediators have been identified as playing important roles in these disease processes. It is our current understanding that the initial inflammatory response aimed at counteracting an underlying injurious event, for unknown reasons, loses the capacity to regulate itself and leads to an exaggerated production of inflammatory mediators that further injures vital organs, with the lungs commonly being among the earliest to be affected (2,3). Tumor necrosis factor (TNF)-α and Interleukin (IL-1) are believed to be the initial triggers of the inflammatory cascade in response to endotoxin or other injurious stimuli (1,2,3). A large number of downstream pro-inflammatory cytokines, chemokines and leukocyte-adhesion factors are then released, followed by the recruitment and activation of inflammatory cells (i.e. macrophages and neutrophils) to the area of injury (2,4,5). Other factors that are activated and contribute to the development of ARDS include coagulation pathway proteins, complement factors, and arachidonic acid metabolites. The recruitment and activation of neutrophils may play an important role due to their release of proteases (i.e., neutrophil elastase) oxidants (i.e., superoxide, myeloperoxidase and hydrogen peroxide), leukotrienes and platelet activating factor (PAF)(4,6,7,8). On the other hand, there are several known endogenous inhibitors of inflammation, including the IL-1 receptor antagonist (IL-1Ra), soluble TNF receptor (sTNFR), autoantibodies against IL-8, and anti-inflammatory cytokines such as IL-10 and IL-11, all of which are believed to regulate the inflammatory and immune response to injury, and may participate in its resolution (4). The duration and severity of ARDS is unpredictable in any given patient, but levels of inflammatory mediators do appear to correlate with mortality (6).

CURRENT TREATMENT MODALITIES AND THEIR LIMITATIONS

Current management of ARDS is based mainly on supportive care and in the prevention of complications. Therapy aimed specifically at the pathogenesis of sepsis and ARDS has been largely unsuccessful. These treatment modalities have included a variety of compounds aimed at blocking or inhibiting the production or effect of known inflammatory mediators. Because of the important role that endotoxin plays in the initiation of the inflammatory cascade in gram-negative sepsis, monoclonal anti-endotoxin antibodies were among the first specific therapies to be developed, but several human clinical trials failed to show a significant survival benefit (9,10,11). A clinical trial using high dose corticosteroids for the treatment of ARDS also failed to significantly affect outcome (12). The non-steroidal anti-inflammatory drug (NSAID), ibuprofen, was studied in a sepsis trial, and even though it had a beneficial effect on some biochemical and clinical parameters, it did not prevent the development of shock or ARDS, nor did it improve survival (13). The inhibition of TNF has been the target of several drugs (i.e. TNF monoclonal and polyclonal antibodies, TNF receptor fusion proteins), but significant beneficial results have not been obtained so far (6,14).

Other therapies that have been considered include IL-1ra, PAF antagonist, pentoxifylline, antioxidants, antiproteases, lipid mediators (PGE_1 and PGE_2), inhaled pulmonary vasodilators (nitric oxide, PGI_2, PGE_2), bradykinin antagonist, and surfactant replacement therapy (6,15). Some of the drugs have already been studied in humans, and even though a few (e.g., the bradykinin antagonist, Deltibant, and the antioxidant oxothiazolidine) have showed a potential benefit, larger prospective, randomized trials are needed to confirm these initial observations (16,17). Since surfactant function is clearly impaired in patients with ARDS, surfactant replacement could be considered as a logical therapeutic option, however its use in adults requires large volumes, which becomes exceedingly costly (4,6).

It is clear that a better understanding of the underlying pathogenetic processes and chronology of events will be important if we are to develop therapies aimed at halting an excessive inflammatory response that characterize the severe forms of the SIRS. One of the limitations of currently available therapies is that they are aimed at the inhibition of a single mediator, which allows for compensatory activation of other mediators, resulting in perpetuation of the inflammatory response. In addition, due to the rapid progression of these diseases there may be an inability to administer the drug soon enough to positively affect outcome. Furthermore, the organs (i.e., liver, lungs, kidneys, etc.) that may play the most critical roles in the

maintenance or exaggeration of the inflammatory response need to be identified and the critical organ(s) should be accessible to the proposed therapy. Finally, it is possible that the lack of effect of the above mentioned drugs is concentration-dependent, so that achieving a beneficial effect in the target organ would require the administration of toxic dose into the systemic circulation.

RATIONALE FOR THE USE OF GENE THERAPY IN ARDS

Most of the initial work in the field of gene therapy has been aimed at using this technology as a therapeutic tool for inherited diseases, especially for cystic fibrosis (CF). Even though much has been learned from the CF experience, the development of a delivery system that can safely result in permanent gene expression is still underway. Two of the more prevalent inherited diseases of the lung, CF and α_1-antitrypsin deficiency, account for a very small fraction of diseases of the lung that affect humans. Therefore, limiting the concept of gene therapy to diseases that are a consequence of the inheritance of a single defective gene would restrict significantly the potential value of this therapy. Furthermore, since the overwhelming majority of diseases of the lungs are acquired, and as we continue to understand the mechanisms of disease in greater detail, we will be able to identify disease pathways that, with the use of gene therapy, can be manipulated in order to restore homeostasis.

As discussed above, ARDS is the result of the hosts' response to an injurious stimulus, and involves a complex interaction between multiple pathogenic factors such as pro-inflammatory cytokines, chemokines, proteases, oxygen radicals and lipid mediators, and protective mechanisms such as anti-inflammatory cytokines, anti-proteases, anti-oxidants and some prostanoids. This complex cascade of events makes it unlikely that the administration of a specific protective cytokine or the inhibition of a pathogenic cytokine would result in a significant change in the course of ALI. On the other hand, a therapeutic tool that could alter the effect of a group of factors would probably have a greater impact in the natural history of the disease.

In addition to the theoretical benefits of a specific therapeutic compound, it is of equal importance to consider the mechanism of action of the agent, its delivery system, its accessibility to the organ or cells of interest, as well as its localization in the intracellular versus extracellular environment. For instance, if a specific protein requires an intracellular localization to be

effective, then the sole administration of that protein to the systemic circulation or the airways would not guarantee its desired effect. This will be better exemplified below when we discuss the effects of α_1-antitrypsin replacement, in which the delivery of the protein product does not have the anti-inflammatory effects seen as when the product is delivered as its gene, in which case the protein is produced intracellularly.

As mentioned above, one of the greater limitations of gene therapy for inherited diseases at the current time is the fact that, regardless of the delivery system used, it has been difficult to obtain permanent expression of the delivered gene. However, DNA therapy for acute diseases requires that the transgene be expressed only transiently. Since one of the difficulties encountered in *in vivo* DNA delivery has been a short duration of expression of the transgene, this requirement could be easily met for the treatment of acute diseases. Another main problem that has been encountered in clinical trials for cystic fibrosis that have used replication-deficient adenoviral vectors has been the development of an acute inflammatory response and a host immune response (18). Other viral vectors, like the adeno-associated viruses, appear to cause less of an inflammatory response, but the transgenes delivered with this vector integrate into the host genome and, hence, theoretically will have permanent expression. This latter characteristic would make them undesirable for the treatment of acute acquired diseases.

The second type of delivery systems are the cationic liposome/plasmid constructs. Liposomes are artificial lipid bilayers designed to translocate drugs or nucleic acids into the cell cytosol via cell-membrane fusion or endocytosis, and, hence, take advantage of the endogenous cellular entry mechanisms to permit efficient delivery of the therapeutic gene (19). Cationic liposome/plasmid delivery systems have some significant advantages for therapy of acute diseases (20). These constructs can deliver functioning transgenes to the lungs following intravenous or airway administration and, delivered to the lungs of experimental animals, either by injection or aerosol, cationic liposome/plasmid complexes appear to be safe (21,22,23,24). Even though no effect on lung mechanics, gas exchange or histology where found in rabbits administered a cationic liposome/plasmid complex, there is evidence that there may be a mild to moderate local inflammatory reaction with the use of this therapy, probably due to immunogenicity to bacterial-derived DNA (21,25). As mentioned above another advantage of this delivery system is the transient expression of the transgene since it does not ordinarily integrate in to the host genome or replicate in mammalian cells (20,26).

We propose two rationales that are based on taking maximum advantage of the current delivery and expression technologies. First, employing genes that encode enzymes may minimize the amount of transgene expression necessary for a therapeutic effect, because the effect is amplified by the intracellular generation of the therapeutic agent. Second, by expressing therapeutic proteins in lung cells where the protein is not normally produced, a local therapeutic effect may be achieved which is not possible by delivering the preformed protein exogenously. We will explain this concept in more detail and with some examples in the next section.

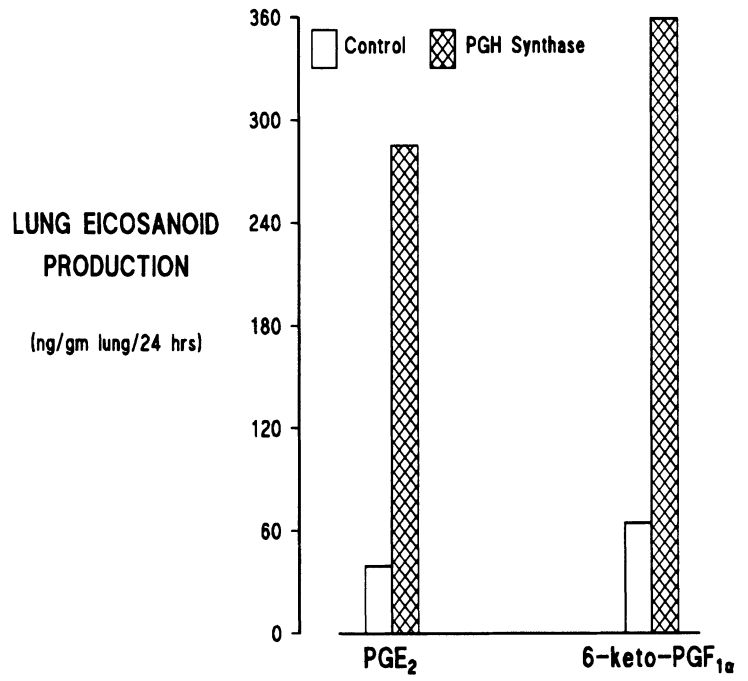

Figure 1. Production of prostaglandin E_2 and prostacyclin (measured as the stable metabolite 6-keto $PGF_{1\alpha}$) by lungs removed from rabbits transfected 24 hours earlier by intravenous delivery of a plasmid containing the COX-1 gene complexed to cationic liposomes. Lung minces were incubated for 24 hours and eicosanoids measured in the supernatant by gas chromatography/mass spectrometry. Control animals were given a plasmid without the transgene. (Reprinted with permission from ref. 27).

CANDIDATE GENES FOR USE IN CRITICAL ILLNESS

The Cyclooxygenase gene

A very good example of using a gene encoding and enzyme is that of the cyclooxygenase (COX) gene. This enzyme catalyzes the production of a host of prostanoids from arachidonic acid, which include PGI_2 and PGE_2, which together have several beneficial (vasodilatory, anti-thrombotic and anti-inflammatory) effects in ALI and other diseases, and thromboxane A_2 (TXA_2), which has vasoconstrictive and pro-thrombotic effects, which are deleterious (27). Fortunately, since COX products are cell specific and endothelial cells only produce PGI_2 and PGE_2, we conducted *in vivo* experiments in rabbits in which we delivered, intravenously, a plasmid containing the human COX-1 gene driven by a CMV promoter complexed to cationic liposomes (27). Rabbits intravenously transfected with the COX-1

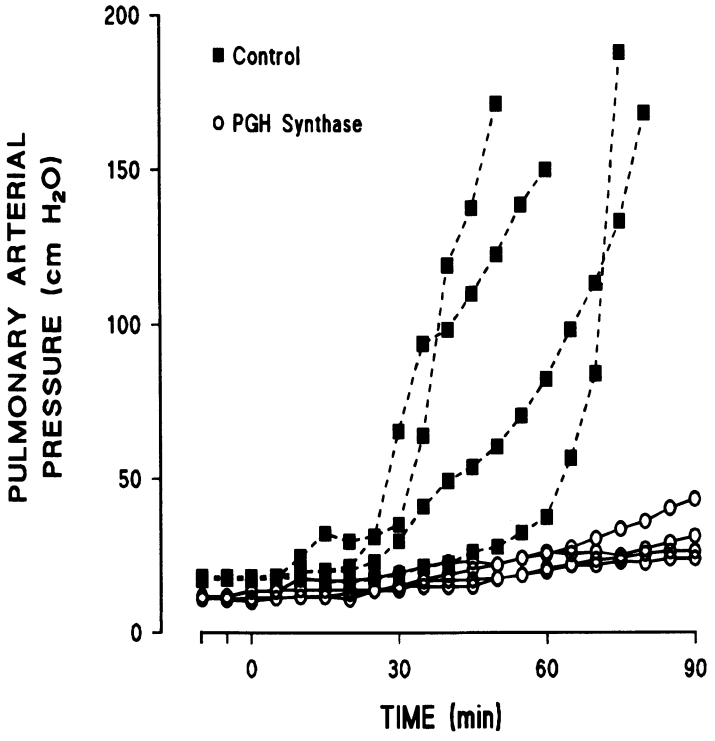

Figure 2. Effects of intravenous transfection, with a plasmid containing the COX-1 gene complexed to cationic liposomes, on the pulmonary vascular response to endotoxin in rabbits. Control animals animals received the same plasmid without the transgene. Animals receiving the COX-1 gene did not show the marked increase in pulmonary vascular resistance typical of the endotoxin response. (Reprinted from ref. 27, with permission).

The α_1-antitrypsin Gene

Transferring genes to cells in the lungs that do not normally express the gene may have unique therapeutic potential because the encoded protein is located at a critical site. Although the α_1-antitrypsin (AAT) protein is not normally produced in respiratory epithelial cells, expression of the normal gene in that cell population might result in locally protective protein concentrations with much less transgene expression than needed to achieve therapeutic serum

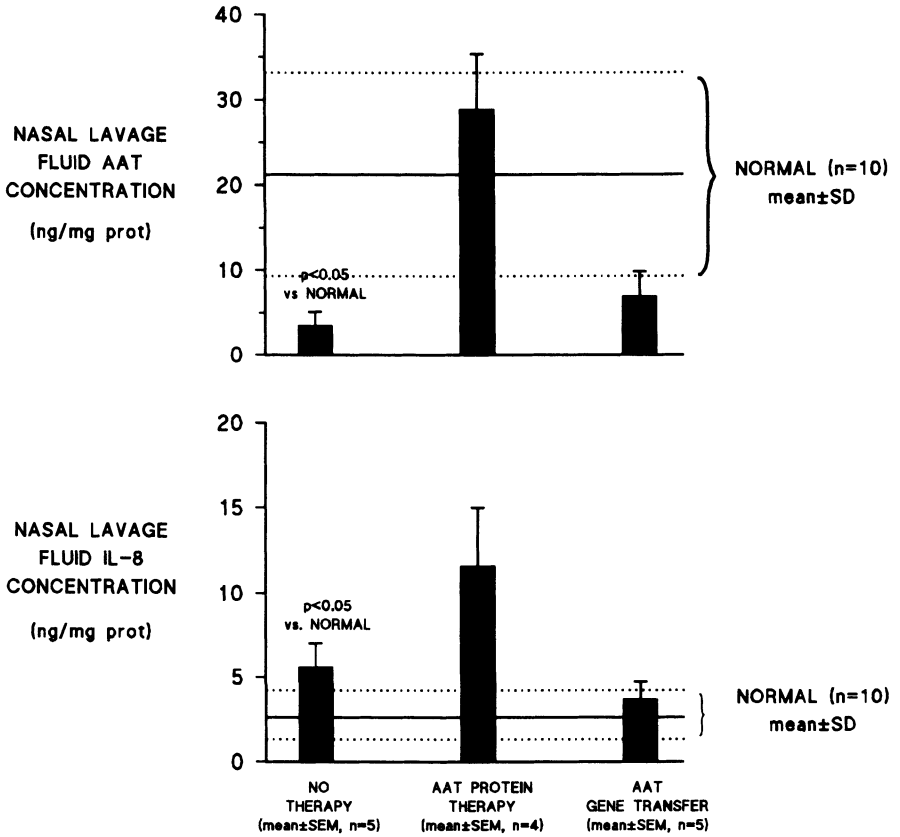

Figure 3. Top: concentrations of AAT normalized to total protein concentrations in nasal lavage fluid (NALF) from patients while receiving no therapy, while receiving weekly intravenous AAT protein therapy, and at the time of peak AAT concentration after transfection with the AAT gene. Means and SD for 10 normal subjects are shown for comparison. Among patients not receiving therapy, AAT concentrations were low compared to normal subjects ($p < 0.05$). AAT protein therapy increased AAT levels to normal. Transfection with the AAT gene increased AAT levels to about one-third of the normal mean. *Bottom*: concentrations of IL-8 normalized to total protein concentration in the same NALF samples for which AAT data are shown (*top*). Means and SD for 10 normal subjects are shown for comparison. Among patients not receiving therapy, IL-8 concentrations were significantly higher than normal ($p < 0.05$). While receiving AAT protein therapy, mean IL-8 levels were higher than while not receiving therapy, but the difference was not significant. In contrast, transfection with the AAT gene decreased IL-8 concentrations to normal ($p < 0.05$ vs. no therapy). (Reprinted from ref. 29, with permission).

concentrations. The rationale for considering AAT gene therapy in ARDS includes its purported anti-inflammatory effects (see below), as well as its anti-protease effect, especially since proteases are thought to play an important role in the pathogenesis of inflammatory diseases (see above) (7). The pathogenesis of both viral infections and inflammation involves proteolytic events, which are associated with tissue destruction and proteolysis of protective proteins, including the AAT protein (7). Low molecular weight antiproteases, which can access the cell interior and possibly other cryptic spaces (i.e., intercellular space), from which large molecules like AAT are excluded, have anti-viral and anti-inflammatory activity. In the right location, AAT could have therapeutic effects that the circulating protein does not. In this case, gene transfer could be considered as a drug delivery system.

The AAT gene has been successfully transfected intravenously and by aerosol to the lungs of rabbits (28). A plasmid containing the AAT gene and a CMV promoter complexed to cationic liposomes resulted in AAT protein expression in pulmonary endothelium following intravenous administration, in alveolar epithelium following aerosol administration, and in the airway epithelium by either route, with expression lasting for at least 7 days (28). In another study done in our laboratories in which pigs were subjected to either intravenous AAT protein administration or intravenous delivery of the AAT gene complexed to cationic liposomes, there was a difference in the compartmental expression of the AAT protein by immunohistochemistry, with the former approach showing AAT localization exclusively in the endothelium, while the latter approach showed AAT localization throughout the vessel wall, as well as in the surrounding parenchyma (unpublished results). Potentially AAT gene therapy could prove useful for patients with acute lung injury.

There is evidence to support that expression of the AAT gene in respiratory epithelial cells has anti-viral and anti-inflammatory effects, while the administration of the AAT protein does not. In an unblinded study, the normal AAT gene was delivered in a plasmid cationic/liposome complex to one nostril of each of five subjects with AAT deficiency, while the other nostril served as control (29). AAT transfection efficiency was confirmed by measuring increased AAT protein concentrations in nasal lavage fluid (NALF) from the transfected, but not from the control nostrils. In addition, reverse-transcriptase-polymerase chain reaction (RT-PCR) was positive for transgene message in all transfected nostrils measured. Furthermore, and more interestingly, AAT transfection was associated with decreased concentrations in NALF of the pro-inflammatory chemokine, IL-8, compared

to baseline (figure 3). This reduction in NALF IL-8 concentration was not seen in the untransfected nostrils as well as in four patients who received intravenous AAT protein, even though they had normal concentrations of AAT in NALF. In another study, using a transformed airway epithelial cell line, which is susceptible to infection with the respiratory syncitial virus (RSV), transfection with plasmid cationic/liposome complex containing the AAT gene inhibited RSV infectivity in this cell line, while the results could not be duplicated be the addition of exogenous AAT protein (29). The above studies suggest that the protein synthesized by transfected cells may have different therapeutic potential than even the same protein delivered exogenously. The anti-inflammatory effects seen in the transfected nasal respiratory epithelium are encouraging and warrant further investigation to assess its applicability to acute lung injury, in which the damaging effects of proteolysis are well documented.

The IL-10 gene

Interleukin 10 is an anti-inflammatory and immunomodulatory cytokine that has been shown to have great potential for the treatment of acute and chronic inflammatory conditions (30). IL-10 inhibits cytokine production by various cell types, and studies using the recombinant form of IL-10 have confirmed its anti-inflammatory effects in animal models of endotoxemia, airway inflammation and autoimmune diseases (31). In humans, the administration of IL-10 has been shown to inhibit the production of cytokines as well as the immune response (32). The recombinant form of IL-10 has been used with some success in humans with steroid-resistant Crohn s disease (33). However, as with the AAT protein, the effects of the *in vivo* adiministration of recombinant IL-10 may be limited due to its short half-life and inability to reach the tissue interstitium (34).

It is, therefore, that IL-10-based gene therapy may prove to be of greater benefit than the sole administration of its protein product. *In vivo* models of lethal endotoxemia have shown that plasmid-mediated IL-10 gene transfer results in reduction of mortality and immune responses in mice (35). In addition, Xing et al. showed that the intramuscular administration to mice of an adenoviral/IL-10 gene complex resulted in increased circulating levels of IL-10, while TNFα and IL-6 levels decreased significantly after endotoxin challenge compared to mice transfected with a control vector (34). Furthermore, endotoxin-induced cytokine gene expression was inhibited in various organs, but more significantly in the lungs (34). The above results support the notion that local over-expression of the IL-10 gene and the release of its protein product into the systemic circulation results in a

beneficial effect at multiple tissue sites (34). Due to its important role in the regulation of the inflammatory cascade IL-10-based gene therapy may be an attractive and powerful therapeutic tool for the treatment of acute illnesses, including ARDS and sepsis syndrome.

Surfactant gene therapy

It is well known that ARDS is associated with abnormalities in the production, composition, and function of surfactant, which probably contributes to alveolar collapse and gas-exchange abnormalities (4). The administration of aerosolized surfactant has proven to be effective in the neonatal form of respiratory distress syndrome (36), but it has not been successful in adults with ARDS (37). It may be possible that the lack of effect of exogenous surfactant in adults is related to an inefficient delivery method or to the high doses required to have a measurable effect in adult lungs (4,6). In addition, administration of large doses of surfactant to adult lungs would result in excessive costs (6).

Therefore, surfactant replacement therapy could be accomplished more effectively by delivering the gene of a surfactant-associated protein (SP) to the airways, which would result in a higher local production of this protein. *In vivo* intratracheal infection of rats with replication-deficient adenoviral vectors containing the DNA for SP-A and SP-B resulted in increased mRNA expression and protein production in lung tissue and bronchoalveolar lavage (BAL)(38,39). Thus, it could be feasible to transfer and express the human surfactant associated protein cDNAs *in vivo* to humans with ARDS, as well as to neonates with the respiratory distress syndrome, as a means of surfactant replacement. This approach could also represent a more cost-effective means of replacing surfactant.

Antioxidant gene therapy

Highly reactive oxygen radicals, including hydrogen peroxide (H_2O_2), the hydroxyl radical (OH) and the superoxide anion, are released by activated inflammatory phagocytes, and are believed to play an important role in the oxidant injury associated with ARDS (4,6,8). The effects of these oxygen radicals are counteracted by elaborate defense mechanisms, which include antioxidant enzyme systems (e.g., superoxide dismutase (SOD), catalase, and the glutathione redox cycle) and various small molecular weight soluble oxidant scavengers (e.g., vitamin E, beta-carotene, vitamin C and uric acid) (6). It is thought that these defenses are overwhelmed in ARDS and, therefore, strategies that could augment the stores of these antioxidant factors could prove to be of great benefit in the treatment of ARDS (6,8).

Several animal models of acute lung injury have shown that gene therapy with antioxidant enzymes is protective against the effects of oxidant-mediated lung injury. Epperly et al. showed that overexpression of a transgene for human manganese SOD delivered intratracheally by either a plasmid liposome or an adenoviral vector resulted in decreased alveolitis and fibrosis after whole lung irradiation (40). Similarly, Danel et al. delivered an adenovirus-encoding SOD and/or catalase intratracheally to rats, and demonstrated a reduction of hyperoxia-induced oxidative lung injury (41). These studies may provide the rationale for designing studies in humans using a gene therapy approach to deliver antioxidant enzymes as therapy for ARDS.

SUMMARY

Septic shock, ARDS and MODS are common problems in critical care units and still account for a large proportion of the morbidity and mortality seen in the ICU. Current management of these conditions is based mainly on supportive care and prevention of complications. Even though overall ICU mortality has decreased during the last two decades, therapy aimed specifically at the pathogenesis of sepsis and ARDS has been largely unsuccessful and mortality rates remain unacceptably high. It is, therefore, important that novel therapies be developed that are capable of reversing the clinical course of critical illnesses. Therapies aimed at inhibiting a variety of inflammatory cytokines have not yielded significant beneficial results, although clinical trials are still underway. Gene-based therapies for critical care illnesses are among the most promising in the horizon, even though their potential benefits are derived from *in vitro* and *in vivo* animal studies. In this chapter we have discussed the rationale for using gene therapy in the treatment of acute illnesses as well as the advantages of delivering genes compared to the delivery of a protein product. Two major advantages of gene-based therapies include the possibility of delivering a gene to cells where the gene product is not normally produced, and, therefore, achieving a local therapeutic effect that is not normally produced by administering the exogenous protein, and second, the use of genes that encode for enzymes that can amplify the production of the therapeutic protein. We have presented available information that demonstrates, in several animal models, that the delivery of therapeutic genes either by replication-deficient adenoviral vectors or by cationic/liposome complexes produces measurable gene expression, as well as increased amounts of its protein, with significant

physiologic improvement seen in the transfected animals. Genes that have been explored as having potential benefit in critical care illnesses, specifically ARDS, include the COX-1, AAT, IL-10, SP-A and SP-B and various antioxidant genes (SOD and catalase). The results seen in animal as well as in preliminary human studies are encouraging and should stimulate our interest to pursue this means of therapy. As we learn more about the role that other inflammatory mediators play in the pathogenesis of critical illnesses we will be able to identify those who may be manipulated by the delivery of appropriate genes and, hence, reverse the progression of these disease processes.

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