CRITICAL CARE

REVIEW

Clinical Review: Gene-based therapies for ALI/ARDS: where are we now?

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Abstract

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) confer substantial morbidity and mortality, and have no specific therapy. The accessibility of the distal lung epithelium via the airway route, and the relatively transient nature of ALI/ARDS, suggest that the disease may be amenable to gene-based therapies. Ongoing advances in our understanding of the pathophysiology of ALI/ARDS have revealed multiple therapeutic targets for gene-based approaches. Strategies to enhance or restore lung epithelial and/or endothelial cell function, to strengthen lung defense mechanisms against injury, to speed clearance of infection and to enhance the repair process following ALI/ARDS have all demonstrated promise in preclinical models. Despite three decades of gene therapy research, however, the clinical potential for gene-based approaches to lung diseases including ALI/ARDS remains to be realized. Multiple barriers to effective pulmonary gene therapy exist, including the pulmonary architecture, pulmonary defense mechanisms against inhaled particles, the immunogenicity of viral vectors and the poor transfection efficiency of nonviral delivery methods. Deficits remain in our knowledge regarding the optimal molecular targets for gene-based approaches. Encouragingly, recent progress in overcoming these barriers offers hope for the successful translation of gene-based approaches for ALI/ARDS to the clinical setting.

Background and context

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) constitute the leading cause of death in pediatric and adult critical care [1]. In the United States alone there are approximately 190,600 cases of ALI/ARDS with a 40% mortality rate, amounting to 75,000 deaths annually [2]. Significant ongoing morbidity, including pulmonary, neuromuscular, cognitive and psychiatric sequelae, is seen in 50 to 70% of ALI/ARDS survivors, and the financial burden on society is considerable [1,3]. There are no specific therapies for ALI/ARDS, and management remains supportive, focusing on protective mechanical ventilation strategies [4], restrictive intravenous fluid management approaches [5], and rescue strategies such as prone positioning [6] or extracorporeal membrane oxygenation [7] for severely hypoxemic patients. These issues underline the need to consider nonconventional therapeutic approaches.

Gene therapy: opportunities in ALI/ARDS

Gene-based therapy involves the insertion of genes or smaller nucleic acid sequences into cells and tissues to replace the function of a defective gene, or to alter the production of a specific gene product, in order to treat a disease. Gene therapy can be classified into germline and somatic gene therapies. Germline approaches modify the sperm or egg prior to fertilization and confer a stable heritable genetic modification. Somatic gene approaches use gene therapy to alter the function of mature cells. Commonly used somatic gene therapy strategies include the overexpression of an existing gene and/or the insertion of smaller nucleic acid sequences into cells to alter the production of an existing gene.

ALI/ARDS may be suitable for gene-based therapies as it is an acute but relatively transient process [8], requiring short-lived gene expression, obviating the need for repeated therapies and reducing the risk of an adverse immunological response. The distal lung epithelium is selectively accessible via the tracheal route of administration, allowing targeting of the pulmonary epithelium [9]. The pulmonary vasculature is also relatively accessible, as the entire cardiac output must transit this circulation. Antibodies that bind antigens selectively expressed on the pulmonary endothelial surface can be complexed to gene vectors to facilitate selective targeting following intravenous administration [10]. It is also possible to use gene-based strategies to target other cells central to the pathogenesis of ALI/ARDS, such as leukocytes and

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fibroblasts [11]. Furthermore, gene-therapy-based approaches offer the potential to selectively target different phases of the injury and repair process. The potential to target specific aspects of the injury and repair processes such as epithelial–mesenchymal transition, fibrosis, fibrinolysis, coagulopathy and oxidative stress with these approaches is also clear.

Current gene-based approaches

Gene therapy requires the delivery of genes or smaller nucleic acid sequences into the cell nucleus using a carrier or vector. The vector enables the gene to overcome barriers to entry into the cell, and to make its way to the nucleus to be transcribed and translated itself or to modulate transcription and/or translation of other genes. Both viral and nonviral vector systems have been developed (Table 1).

Viral vector-delivered gene therapy

Viral vectors are the most effective and efficient way of getting larger nucleic acid sequences, particularly genes, into cells (Table 1). The viral genome is modified to remove the parts necessary for viral replication. This segment is then replaced with the gene of interest – termed a transgene – coupled to a promoter that drives its expression. The modified genome is then encapsulated with viral proteins. Following delivery to the target site, the virus binds to the host cell, enters the cytoplasm and releases its payload into the nucleus (Figure 1). The size of transgene that can be used depends on the capsid size. A number of different viral vectors have been used in preclinical lung injury studies to date.

Adenoviral vectors

Adenoviruses have double-stranded DNA genomes, have demonstrated promise in preclinical models [12,13] and are well tolerated at low to intermediate doses in humans [14,15]. Advantages include their ease of production, the high efficiency at which they can infect the pulmonary epithelium [14,16] and that they can deliver relatively large transgenes. A disadvantage of adenoviruses is their immunogenicity, particularly in repeated doses [14]. Newer adenoviral vectors, in which much of the immunogenicity has been removed, hold promise [17]. While adenovirus-mediated gene transfer in the absence of epithelial damage is relatively inefficient [18], this may be less of a problem in ALI/ARDS that is characterized by widespread epithelial damage.

Adeno-associated virus vectors

Adeno-associated viruses (AAVs) are single-stranded DNA parvoviruses that are replication deficient [19]. A substantial proportion of the human population has been exposed to AAVs but the clinical effects are unknown. AAV vectors have a good safety profile, and are less immunogenic compared with other viruses, although antibodies do develop against AAV capsid proteins that can compromise repeat administration. AAV vectors can insert genes at a specific site on chromosome 19. The packaging capacity of the virus is limited to 4.7 kb, restricting the size of the transgene that can be used. AAVs are less efficient in transducing cells than adenoviral vectors. Successful AAV vector gene transfer has been demonstrated in multiple lung cell types including lung progenitor cells, in both normal and naphthalene-induced ALI lungs [20]. AAV serotypes have specific tissue tropisms, due to different capsid proteins that bind to specific cell membrane receptors. AAV-5 [21] and AVV-6 [22] exhibit enhanced tropism for the pulmonary epithelium [21,22]. AAVs can transduce nondividing cells and result in long-lived transgene expression. AAV vectors have been used in clinical trials in cystic fibrosis patients, underlining their safety profile [23,24].

Lentivirus vectors

These RNA viruses can transfect nondividing cells such as mature airway epithelial cells [25]. The virus stably but randomly integrates into the genome and expression is likely to last for the lifetime of the cell (~100 days). The transgene can be transmitted post mitosis, and there is also a risk of tumorigenesis if the transgene integrates near an oncogene. The development of leukemias in children following gene therapy for severe combined immunodeficiency highlights this risk [26,27]. While lentiviral vectors may be useful to correct a gene deficiency associated with increased risk of ALI, the long-lived gene expression of lentiviral delivered genes may be more suitable for chronic diseases than for ALI/ARDS.

Nonviral gene-based strategies

Nonviral delivery systems, while generally less efficient than viral vectors in transfecting the lung epithelium, are increasingly used to deliver smaller DNA/RNA molecules (Table 1). Strategies include the use of DNA–lipid and DNA–polymer complexes and naked DNA/RNA oligonucleotides, such as siRNA [28], decoy oligonucleotides [29] and plasmid DNA [30]. Nonviral delivery systems are less immunogenic than viral vector-based approaches, and can be generated in large amounts at relatively low cost.

Plasmid transfer

Plasmid vectors are composed of closed circles of double-stranded DNA. As naked and plasmid DNA contain no proteins for attachment to cellular receptors, there is no specific targeting to different cell types and thus it is essential that the DNA is placed in close contact with the desired cell type. These limitations make this approach less relevant clinically.

Table 1. Gene therapy approaches used in preclinical ALI/ARDS models

Approach	Advantages	Disadvantages	Examples
Viral vector-delivered gene therapy			
Adenoviral vectors (dsDNA genome)	Relatively easily produced Efficiently transfect lung epithelium [14,16] Can deliver larger genes Well tolerated in lower doses [1,3]	Immunogenic [14]	Adenoviral transfer of genes for a surfactant enzyme [49], angiopoietin-1 [51], HSP-70 [52], apolipoprotein A-1 [53], and Na^+,K^+-ATPase pump [55] genes attenuate experimental ALI Adenoviral delivery of IL-10 gene attenuates zymosan ALI at low doses, but is harmful at high doses [58]
Adeno-associated virus vectors (ssDNA genome)	Good safety profile; less immunogenic Inherently replication deficient AAV-5 and AAV-6 lung epithelial tropism [10,11] Transduce nondividing cells Long-lived gene expression Used in clinical trials for CF [12,13]	Limited transgene size Difficult to produce in large quantities	AAV vector gene transfer demonstrated in multiple lung cell types including progenitor cells in both normal lungs and following naphthalene-induced ALI [20]
Lentivirus vectors (RNA genome)	Transduce nondividing cells [25] Integrate stably but randomly into the genome	Oncogenesis risk due to integration into genome [26,27]	Lentiviral transfer of shRNA to silence CD36 gene expression suppresses silica-induced lung fibrosis in the rat [35]
Nonviral gene-based strategies			
Plasmid transfer (closed dsDNA circles)	Easily produced at low cost	No specific cell targeting Very inefficient	Electroporation-mediated gene transfer of the Na^+,K^+-ATPase rescues endotoxin-induced lung injury [60]
Nonviral DNA complexes (lipoplexes or polyplexes)	Complexes protect DNA Complexes facilitate cellular targeting [31]	Less efficient than viral vectors	Cationic lipid-mediated transfer of the Na^+,K^+-ATPase gene ameliorated high-permeability pulmonary edema [59] Lipoplex-delivered IL-10 gene decreased CLP-induced ALI [61] Systemic cationic polyethylenimine polyplexes incorporating indoleamine-2,3-dioxygenase decreased ischemia–reperfusion ALI [62]
DNA and RNA oligonucleotides (siRNA, shRNA, decoy oligonucleotides)	Easily produced at low cost Smaller molecules that can easily enter cells Target regulation of specific genes	No specific cell targeting	Specific siRNAs reduce inflammation-associated lung injury in humans [33] and in animal models [28,34] shRNA-based approaches have reduced lung injury in animal models [35,36]
Cell-delivered gene therapy			
Mesenchymal stem/stromal cells	Systemic or intrapulmonary delivery Strategy used in human studies [41]	Relatively expensive	MSCs expressing angiopoeitin-1 attenuate endotoxin-induced ALI [40] Bone marrow stem cells expressing keratinocyte growth factor via an inducible lentivirus protects against bleomycin-induced lung injury [66]
Fibroblasts	Systemic delivery Less expensive		Fibroblasts expressing angiopoeitin-1 attenuate endotoxin induced ALI [40]

AAV, adeno-associated virus; ALI, acute lung injury; ARDS, acute respiratory distress syndrome; CF, cystic fibrosis; CLP, cecal ligation and puncture; dsDNA, double-stranded DNA; IL, interleukin; MSC, mesenchymal stem/stromal cell; shRNA, small hairpin RNA; siRNA, small inhibiting RNA; ssDNA, single-stranded DNA.

Nonviral DNA complexes

The therapeutic DNA is held within a sphere of lipids, termed a lipoplex, or within a sphere of polymers, such as polyethyleneimine, termed a polyplex. Lipoplexes and polyplexes act to protect the DNA, facilitate binding to the target cell membrane and also trigger endocytosis of the complex into the cell, thereby enhancing gene expression. These systems can be modified to include a targeting peptide for a specific cell type, such as airway epithelial cells [31]. These complexes efficiently and safely transfect airway epithelial cells [31], and they have demonstrated promise in human studies [32].

DNA and RNA oligonucleotides

siRNAs are dsRNA molecules of 20 to 25 nucleotides that can regulate the expression of specific genes. Specific siRNAs reduce inflammation-associated lung injury in

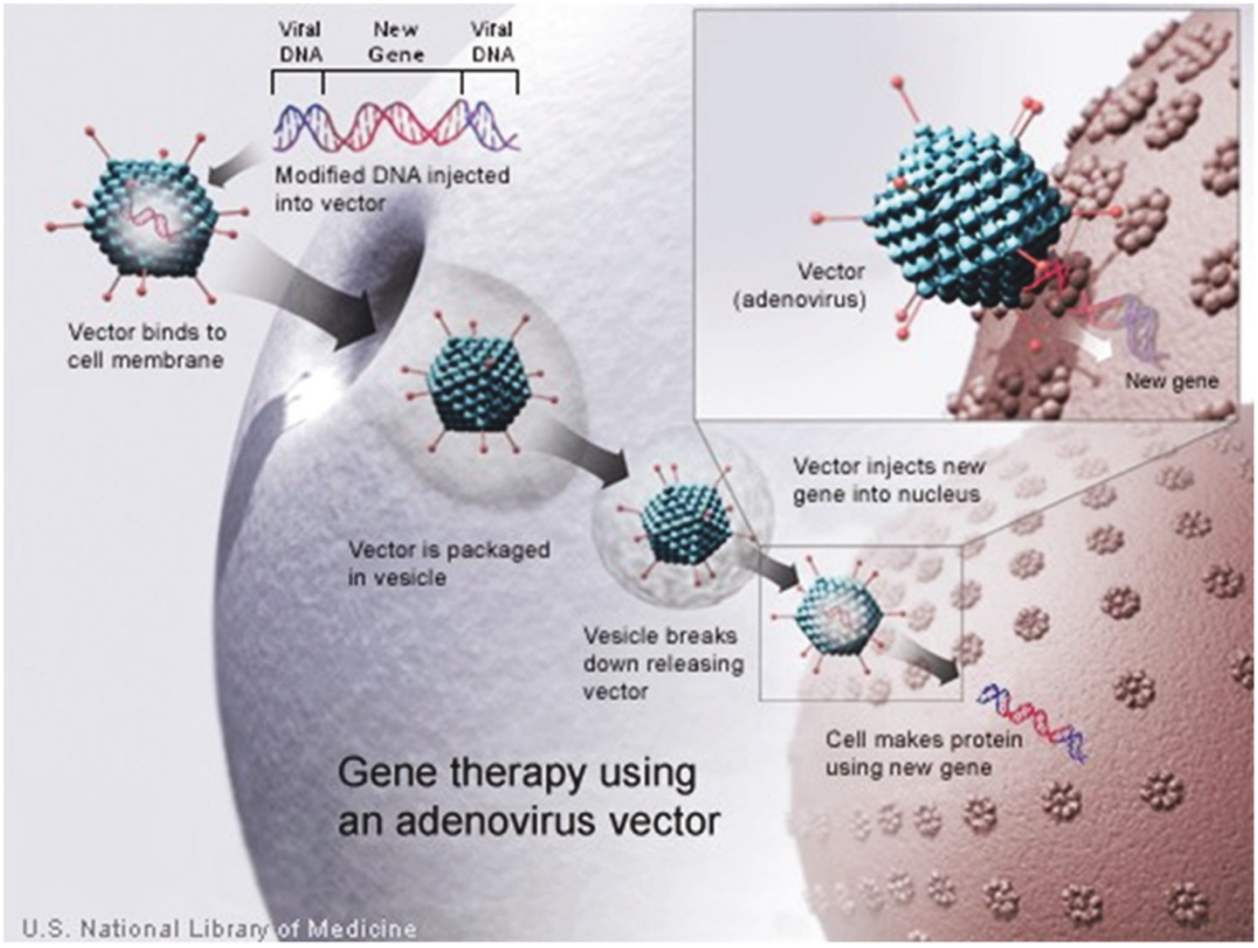

Figure 1. Schematic diagram of the mechanisms involved in viral vector-mediated gene therapy. The adenoviral vector encoding a new gene of interest binds to a cell membrane, becoming internalized within a vesicle, is subsequently released from the vesicle and is transported towards the nucleus, where it releases its genetic material, which is subsequently transcribed and translated to produce the therapeutic protein. This image is a work of the National Institutes of Health, part of the United States Department of Health and Human Services, and is in the public domain.

humans [33] and in animal models [28,34]. shRNA is a single strand of RNA that, when introduced into the cell, is reverse transcribed and integrated into the genome, becoming heritable. During subsequent transcription, the sequence generates an oligonucleotide with a tight hairpin turn that is processed into siRNA. shRNAs have reduced lung injury in animal models [35,36]. Decoy oligonucleotides are double-stranded DNA molecules of 20 to 28 nucleotides, which bind to specific transcription factors to reduce expression of targeted genes, and have been successfully used in animal models [37,38].

Cell-delivered gene therapy

An alternative approach is to use systemically delivered cells to deliver genes to the lung. This approach has been used to enhance the therapeutic potential of stem cells – such as mesenchymal stem/stromal cells, which demonstrate promise in preclinical ALI/ARDS models [39]. Fibroblasts have also been used to successfully deliver genes to the lung to attenuate ALI [40]. Preliminary data from a clinical trial in pulmonary hypertension show that endothelial progenitor cells overexpressing endothelial nitric oxide synthase (*NOS3*) decrease pulmonary vascular resistance [41], highlighting the potential of cell-delivered gene therapy for ALI/ARDS.

Delivery of vector to the lung

Airway delivery

Nebulization of genetic material into the lung is effective [42], safe and well tolerated [32,43,44]. The integrity of AAV vectors [9,43] and adenoviral virus vectors [44] are maintained post nebulization, as are cationic lipid vectors [32] and DNA and RNA oligonucleotides [45]. A number of gene therapy clinical trials have utilized nebulization

to deliver the transgene to the lung [23,43], but without clear clinical benefit to date [43,44].

Intravascular delivery

Intravascular delivery approaches target the lung endothelium. These approaches have been successfully used in preclinical studies of cell-based gene therapies [39,40], and also with vectors that incorporate components such as antibodies to target antigens on the lung endothelium [10].

Barriers to effective gene therapy in ARDS

Successful gene-based therapies require the delivery of high quantities of the gene or oligonucleotide to the pulmonary epithelial or endothelial surface, require efficient entry into the cytoplasm of these large and insoluble nucleic acids, which then have to move from the cytoplasm into the nucleus, and activate transcription of its product. Multiple barriers exist that hinder this process, not least the natural defense mechanisms of the lung, and additional difficulties that exist in transducing the acutely injured lung (Table 2). Limitations regarding delivery technologies and deficiencies in our knowledge regarding the optimal molecular targets also reduce the efficacy of these approaches.

Table 2. Technical challenges to gene-based therapies for ALI/ARDS models

Pulmonary defense mechanisms against inhaled particles
Airway mucus and epithelial lining fluid
Glycocalyceal barrier
Tight intercellular epithelial junctions
Limited endocytosis at luminal surface
Difficulties in transducing the acutely injured lung
Loss of alveolar epithelium
Pulmonary edema
Collapsed and/or consolidated alveoli
Bronchial plugging by mucus and debris
Limitations of vector systems
Imunogenicity of viral vectors particularly in repeated doses
Limitations regarding transgene size
Limited transfection efficiency of nonviral vectors
Knowledge deficits regarding the optimal molecular targets

ALI, acute lung injury; ARDS, acute respiratory distress syndrome.

Pulmonary defense mechanisms

The lung has evolved effective barriers to prevent the uptake of any inhaled foreign particles [46]. While advantageous in minimizing the potential for uptake of external genetic material (for example, viral DNA), these barriers make it more difficult to use gene-based therapies in the lung. Barriers to entry of foreign genetic material into the lung include airway mucus and the epithelial lining fluid, which traps and clears inhaled material. The glycocalyceal barrier hinders contact with the cell membrane, while the tight intercellular epithelial junctions and limited luminal endocytosis further restrict entry of foreign material into the epithelial cells.

Difficulties transducing the injured lung

Transducing the acutely injured lung may be difficult, due to the presence of pulmonary edema, consolidated or collapsed alveoli, and additional extracellular barriers such as mucus. Gene-based therapies targeted at the pulmonary epithelium may be less effective where there is extensive denudation of the pulmonary epithelium, as may occur in primary ARDS. Encouragingly, there is some evidence to suggest that ALI may not substantially impair viral gene transfer to the alveolar epithelium [47].

Limitations of vector systems

The key limitation of nonviral vector approaches has been their lack of efficiency in mediating gene transfer and transgene expression in the airway epithelium. Viral vectors are immunogenic, due to the protein coat of the viral vector, and the immune response is related to both vector dose and number of administrations. The potential to limit administration to a single dose in ALI/ARDS may reduce this risk. However, the development of an inflammatory response resulting in death following administration of a first-generation adenoviral vector highlights the risks involved [48]. Additional limitations of viral vectors include transgene size, which is limited by the size of the capsid that encloses the viral genes.

Insights from preclinical ALI/ARDS studies

The therapeutic potential of gene therapy for ALI/ARDS is underlined by a growing body of literature demonstrating efficacy in relevant preclinical models. In considering the clinical implications of these studies, it is important to acknowledge that animal models of ARDS do not fully replicate the complex pathophysiological changes seen in the clinical setting. This is highlighted by the fact that many pharmacologic strategies demonstrating considerable promise in preclinical studies were later proven ineffective in clinical trials. Nevertheless, these studies provide insights into the clinical potential of these strategies.

Studies using viral vectors

Adenovirus-mediated transfer of a gene that enhances surfactant production improves lung function and confers resistance to *Pseudomonas aeruginosa* infection (Figure 2) [49]. Adenovirus-delivered superoxide dismutase and catalase genes protected against hyperoxic-induced,

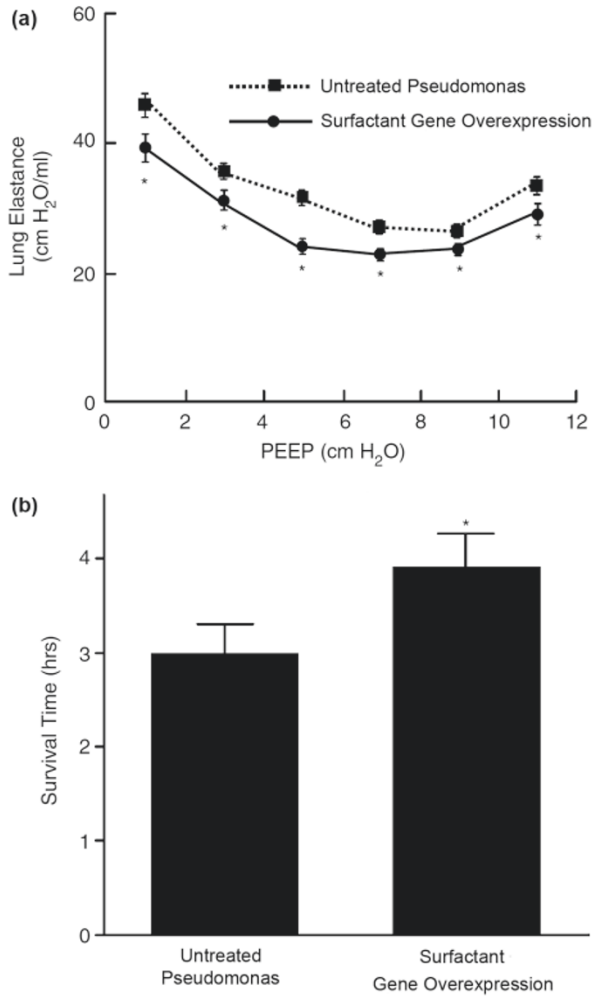

Figure 2. Adenovirus-mediated gene transfer enhances surfactant production, improves lung function and confers resistance to infection. Intratracheal delivery of adenovirus encoding a modified surfactant enzyme enhances lung production of surfactant, and **(a)** attenuated the decrement in lung elastance and **(b)** increased the survival time in mice infected with *Pseudomonas aeruginosa*. PEEP, positive end-expiratory pressure. Reproduced with permission from The Nature Publishing Group [49].

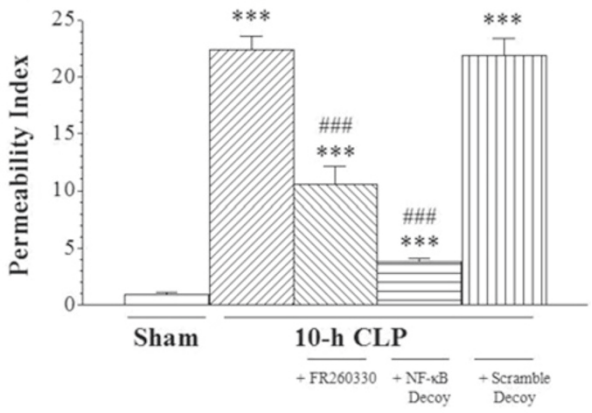

Figure 3. NF-κB decoy oligodeoxynucleotides prevent acute lung injury in mice with cecal ligation and puncture-induced sepsis. The animals were subjected to sham operation (control), sepsis (10 hours after cecal ligation and puncture (CLP)), sepsis with the NF-κB pharmacologic inhibitor FR260330, and sepsis with transfection of NF-κB decoy or its scrambled form. Transpulmonary flux of radiolabeled albumin was used to assess changes in lung permeability. ***P <0.001 compared with the sham-operated control group. ###P <0.001 compared with the CLP group without treatment. Reproduced with permission from The American Society for Pharmacology and Experimental Therapeutics [37].

but not ischemia–reperfusion-induced, lung injury [50]. More recent studies have demonstrated the therapeutic potential of overexpression of a number of genes, including angiopoietin-1 [51], HSP-70 [52], apolipoprotein A-1 [53], defensin β2 [54] and the Na^+,K^+-ATPase pump [55]. In contrast, overexpression of IL-1β can directly cause ALI [56], while overexpression of suppressor of cytokine signaling-3 worsens immune-complex-induced ALI [57]. Intriguingly, intratracheal administration of adenoviral vector incorporating IL-10, prior to zymosan-induced lung injury, improved survival at a lower dose but was ineffective and even harmful at higher doses [58].

Studies using nonviral vectors

An early murine study demonstrated that cationic lipid-mediated transfer of the Na^+,K^+-ATPase gene ameliorated high-permeability pulmonary edema [59]. Electroporation-assisted gene transfer of plasmids encoding for Na^+,K^+-ATPase reverses endotoxin-induced lung injury [60]. The lipoplex-delivered IL-10 gene decreased lung and systemic organ injury induced by cecal ligation and puncture in mice [61]. Systemically administered cationic polyethylenimine polyplexes incorporating indoleamine-2,3-dioxygenase transduced pulmonary endothelial cells and decreased lung ischemia–reperfusion injury [62].

Studies using DNA and RNA oligonucleotides

NF-κB decoy oligonucleotides, incorporated into viral vectors, attenuate systemic sepsis-induced lung injury when administered intravenously (Figure 3) [37]. In animal models, both intratracheal [34,63] and intravenously [29,64] administered siRNA successfully silence their target genes. shRNA-based approaches have been used to suppress silica-induced lung fibrosis [35] and to ameliorate lung ischemia–reperfusion-induced lung injury [36]. More recently, aerosolization of siRNA that targets respiratory syncytial virus viral replication was safe and potentially effective in patients post lung transplant with respiratory syncytial virus infection [33], clearly illustrating the therapeutic potential of these approaches for ALI/ARDS.

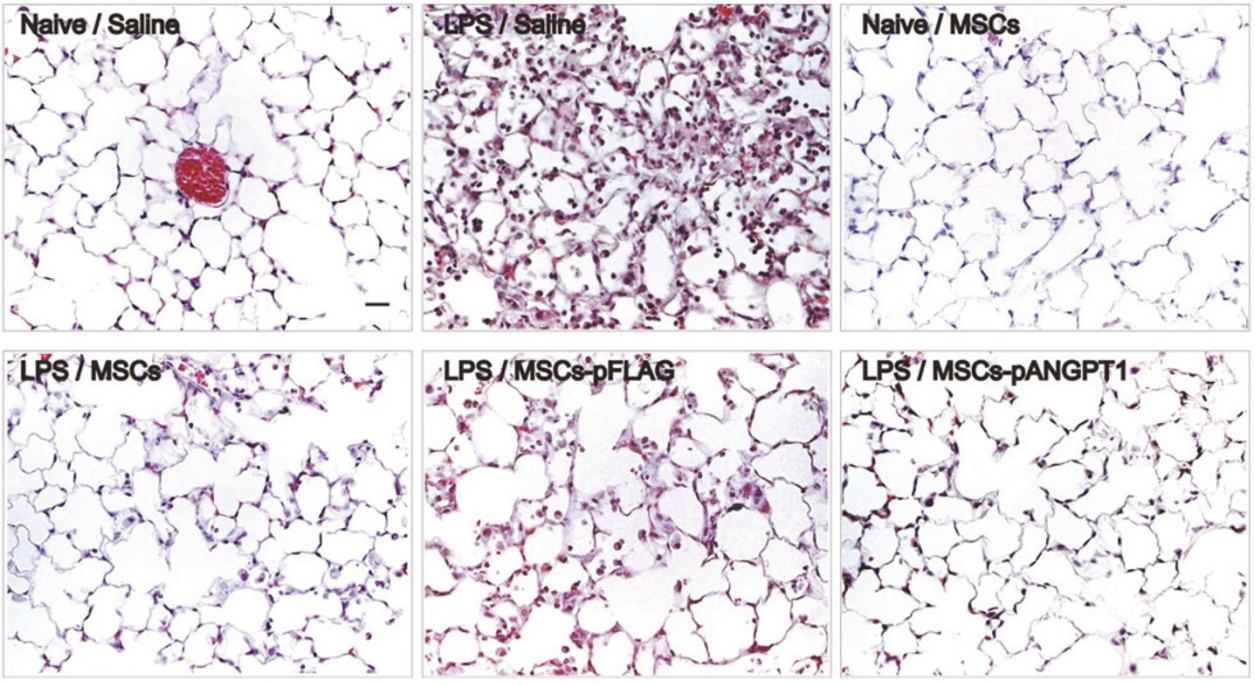

Figure 4. Gene-based therapies may be combined with stem cells to enhance their effect. Representative histologic images of lung sections demonstrate that modification of mesenchymal stem/stromal cells (MSCs) to overexpress angiopoeitin-1 (LPS/MSCs-pANGPT1) enhanced its efficacy in attenuating endotoxin-induced lung injury (LPS/Saline) compared with MSCs alone (LPS/MSCs) or MSCs overexpressing a nonfunctional gene (LPS/MSCs-pFLAG). Reproduced with permission from The Public Library of Science [39].

Studies using cell-delivered gene therapy

Mei and colleagues enhanced the efficacy of mesenchymal stem/stromal cells in endotoxin-induced ALI by transducing them to overexpress angiopoeitin-1 (Figure 4) [39]. Mesenchymal stem/stromal cells overexpressing IL-10 decreased alveolar infiltration of CD4 and CD8 T cells following lung ischemia–reperfusion injury [65]. Bone marrow stem cells expressing keratinocyte growth factor attenuate bleomycin-induced lung injury [66]. Nonstem cells can also be used to deliver genes to the injured lung [67]. Fibroblasts overexpressing angiopoeitin-1 attenuate endotoxin-induced lung injury [40], while fibroblasts overexpressing vascular endothelial growth factor and endothelial nitric oxide synthase can attenuate or even reverse endotoxin-induced ALI [68].

Gene-based therapies for ALI/ARDS: future directions

Advances in the identification of therapeutic targets, improvements in viral and nonviral vector technologies, and regulation of gene-based therapies by temporal and spatial targeting offer the potential to translate the therapeutic promise of gene-based therapies for ALI/ARDS to the clinical setting (Table 3).

Better viral vectors

Viral vectors remain the focus of intensive research to optimize their efficiency, to minimize their immunogenicity and to enhance their tissue specificity [19,31,69,70]. Strategies to develop less immunogenic vectors have focused on modifying the naturally occurring proteins in the viral coat [71]. Much research has been devoted to searching and characterizing both naturally occurring [71] and engineered capsid variants from mammalian species [72]. Capsid protein modification has also been used to enhance tissue specificity [70]. Envelope protein pseudotyping involves encapsulating the modified genome from one virus, such as simian immunodeficiency virus, with envelope proteins from another virus, such as vesicular stomatitic virus. This encapsulation can enhance the therapeutic potential of viral vectors, by combining the advantages of one viral genome (for example, bigger payload or site-specific integration) with the tissue tropism of another virus.

Better nonviral vectors

Strategies to enhance the effectiveness of the lipoplexes used to deliver plasmids and other DNA/RNA oligonucleotides involve manipulation of the lipoplex lipid content and the use of targeting peptides. The choice of lipid influences expression efficiency by enhancing release of the genetic material within the target cell [73,74]. Targeting peptides increases transfection efficiency by directing the lipid to a particular cell membrane or cell type [31]. Physical methods of plasmid delivery

Table 3. Future directions for gene-based therapies

Viral vectors
Capsid protein modification to reduce immunogenicity [71]
Capsid protein modification to enhance tissue specificity [70]
Envelope protein pseudotyping
Nonviral vectors
Manipulation of lipoplex lipid content to enhance cellular uptake [73,74]
Use of targeting peptides on lipoplexes and polyplexes [31]
Strategies to enhance gene transfer; for example, electroporation, ultrasound, gene gun delivery
Gene expression strategies
Modifying transgene DNA to eliminate bacterial motifs [75,76]
Development of high-efficiency tissue-specific promoters [77-80]
Development of promoters that regulate gene expression [83]
Enhanced therapeutic targeting
Nebulization technologies [9]
Strategies to target the pulmonary endothelium [10]
Improved cellular uptake of vector
Surface active agents to enhance vector spread [84]
Reduce ubiquitination of viral capsid proteins [85]
Better therapeutic targets
Enhancement or restoration of lung epithelial and/or endothelial cell function [86]
Strengthening lung defense mechanisms against injury [87]
Speeding clearance of inflammation and infection
Enhancement of the repair process following ALI/ARDS [88].

ALI, acute lung injury; ARDS, acute respiratory distress syndrome.

such as electroporation [60] and ultrasound can enhance gene transfer by bringing the plasmid DNA into closer proximity with the cell membrane and/or causing temporary disruption of the cell membrane. Other physical methods can also be used to increase *in vivo* gene transfer, including pressurized vascular delivery, laser, magnetic fields and gene gun delivery. These systems enable plasmid-based gene delivery to reach efficiencies close to that achieved with viral vectors.

Enhanced gene expression strategies

Successful gene therapy relies upon being able to target the injury site, and to control the duration and levels of gene expression. Modifying the transgene DNA to exclude nonmethylated CpG motifs, typical of bacterial DNA, decreases the immune response and may increase transgene expression [75,76]. High-efficiency tissue-specific promoters may improve the efficiency and specificity of transgene expression. Lung-specific promoters include surfactant promoters [77] such as the surfactant protein C promoter [78], a ciliated cell-specific promoter FOXJ1 [79], the cytokeratin 18 promoter [80], and the Clara cell 10-kDa protein [78]. Promoters can also be used to target a specific phase of illness, switching on when required to produce an effect at the optimal time point.

A related approach is the development of promoters that allow for transfected genes to be turned on and off. Currently, the tetracycline-dependent gene expression vector [81] is the most widely used regulated system as it has a good safety profile. Tetracycline is rapidly metabolized and cleared from the body, making it an ideal drug to control gene expression. However, the potential for an activator such as tetracycline to modulate the lung injury should be borne in mind. New-generation transactivators, with no basal activity and increased sensitivity, have now been developed [82]. In an ARDS context, conditional regulation of gene expression by the combined use of a lung-specific promoter and the tetracycline-dependent gene expression system may be a useful approach [83].

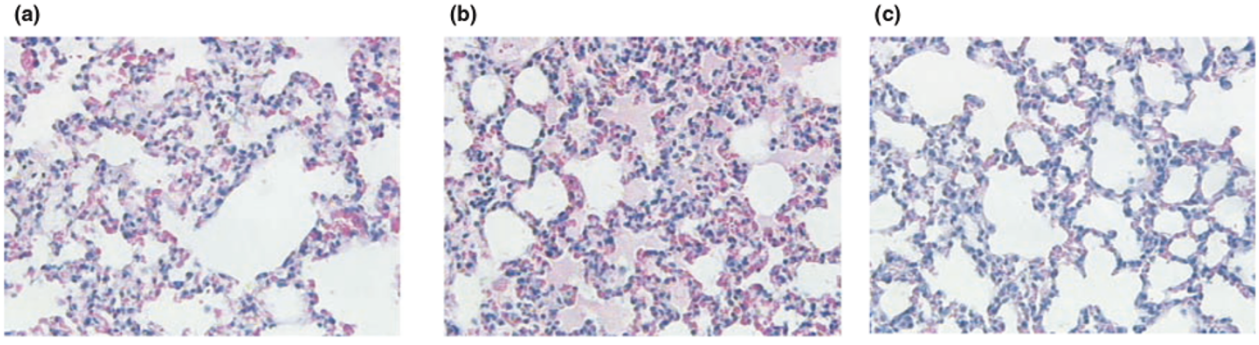

Figure 5. Therapeutic potential of selectively targeting the pulmonary endothelium with systemically delivered gene-based therapies. The antioxidant enzyme catalase was conjugated with antibodies to the adhesion molecule PECAM. The anti-PECAM/catalase conjugate, but not a nonspecific IgG/catalase conjugate, bound specifically to the pulmonary endothelium, and attenuated hydrogen peroxide (H_2O_2) injury. **(a)** H_2O_2 produces a severe lung injury, **(b)** which is not attenuated by the nonspecific IgG/catalase conjugate. **(c)** In contrast, the anti-PECAM/catalase conjugate does attenuate lung injury. Reproduced with permission from the American Physiological Society [10].

Table 4. Key points regarding gene-based therapies for ALI/ARDS

ALI/ARDS may be amenable to gene-based therapies
Ongoing advances in our understanding of the pathophysiology of ALI/ARDS have revealed multiple therapeutic targets for gene-based approaches
Numerous gene-based approaches have demonstrated promise in relevant preclinical models
The clinical potential for gene-based approaches to ALI/ARDS remains to be realized
Multiple barriers exist to successful gene-based approaches for ALI/ARDS
A greater understanding of the molecular mechanisms underlying injury and repair in ALI/ARDS, coupled with improvements in gene-based approaches, offer hope for ALI/ARDS

ALI, acute lung injury; ARDS, acute respiratory distress syndrome.

Enhanced therapeutic targeting

An advantage of gene-based strategies is the ability to target specific cells within an organ; for example, the epithelial cells of the lung. Novel nebulization technologies, which facilitate the delivery of large quantities of undamaged vector to the distal lung, demonstrate considerable promise in this regard [9]. Alternative approaches to spatial targeting include targeting specific receptors that are plentiful on the target cell to increase transfection efficiency. An interesting development in this regard is the targeting of systemically administered therapies to the pulmonary endothelium using antibodies to proteins expressed preferentially on these cells (Figure 5) [10]. In these studies, the antioxidant enzyme catalase was conjugated with antibodies to the adhesion molecule PECAM, which is widely expressed on pulmonary endothelial cells, and to a nonspecific IgG antibody. The anti-PECAM/catalase conjugate, but not the IgG/catalase conjugate, bound specifically to the pulmonary endothelium and attenuated hydrogen peroxide injury.

Improved cellular uptake of vector

Specific strategies have been developed to maximize uptake of vector into alveolar epithelial cells. It is possible to enhance lung transgene expression with the use of surface-active agents such as perflurocarbon, which enhances the spread of vector and mixing within the epithelial lining fluid [84]. Agents that reduce ubiquitination of AAV capsid proteins following endocytosis, such as tripeptide proteasome inhibitors, dramatically augment (>2,000-fold) AAV vector transduction in airway epithelia [85].

Better therapeutic targets

Ultimately, the success or failure of gene-based therapies for ALI/ARDS is likely to rest on the identification of better gene targets. Ongoing advances in our understanding of the pathophysiology of ALI/ARDS continue to reveal novel therapeutic targets for gene-based approaches. Promising potential approaches include strategies to enhance or restore lung epithelial and/or endothelial cell function [86], to strengthen lung defense mechanisms against injury [87], to speed clearance of inflammation and infection, and to enhance the repair process following ALI/ARDS [88].

Summary and conclusions

ALI/ARDS may be a particularly suitable disease process for gene-based therapies (Table 4). This is supported by increasing evidence from relevant preclinical ARDS models for the efficacy of gene-based therapies that enhance or restore lung epithelial and/or endothelial cell function, strengthen lung defense mechanisms against injury, speed resolution of inflammation and infection, and enhance the repair process following ALI/ARDS. Despite this promising preclinical evidence, the potential for gene based approaches to ALI/ARDS in the clinical setting remains to be realized. Multiple barriers exist to the successful use of gene-based therapies in the lung, which limit the efficacy of these approaches. Future research approaches should focus on overcoming these barriers, by developing more effective and less immunogenic vector delivery systems, developing strategies to focus gene expression on specific injury zones of the lung for defined time periods, and identifying better molecular targets that can take advantage of these potentially very powerful therapeutic approaches.

Abbreviations

AAV, adeno-associated virus; ALI, acute lung injury; ARDS, acute respiratory distress syndrome; IL, interleukin; NF, nuclear factor; shRNA, small hairpin RNA; siRNA, small interfering RNA.

Competing interests

The authors declare that they have no competing interests.

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