Gene Therapy in Cystic Fibrosis*

Terence R. Flotte, MD; and Beth L. Laube, PhD

Theoretically, cystic fibrosis transmembrane conductance regulator (CFTR) gene replacement during the neonatal period can decrease morbidity and mortality from cystic fibrosis (CF). In vivo gene transfers have been accomplished in CF patients. Choice of vector, mode of delivery to airways, translocation of genetic information, and sufficient expression level of the normalized CFTR gene are issues that currently are being addressed in the field. The advantages and limitations of viral vectors are a function of the parent virus. Viral vectors used in this setting include adenovirus (Ad) and adeno-associated virus (AAV). Initial studies with Ad vectors resulted in a vector that was efficient for gene transfer with dose-limiting inflammatory effects due to the large amount of viral protein delivered. The next generation of Ad vectors, with more viral coding sequence deletions, has a longer duration of activity and elicits a lesser degree of cell-mediated immunity in mice. A more recent generation of Ad vectors has no viral genes remaining. Despite these changes, the problem of humoral immunity remains with Ad vectors. A variety of strategies such as vector systems requiring single, or widely spaced, administrations, pharmacologic immunosuppression at administration, creation of a stealth vector, modification of immunogenic epitopes, or tolerance induction are being considered to circumvent humoral immunity. AAV vectors have been studied in animal and human models. They do not appear to induce inflammatory changes over a wide range of doses. The level of CFTR messenger RNA expression is difficult to ascertain with AAV vectors since the small size of the vector relative to the CFTR gene leaves no space for vector-specific sequences on which to base assays to distinguish endogenous from vector-expressed messenger RNA. In general, AAV vectors appear to be safe and have superior duration profiles. Cationic liposomes are lipid-DNA complexes. These vectors generally have been less efficient than viral vectors but do not stimulate inflammatory and immunologic responses. Another challenge to the development of clinically feasible gene therapy is delivery mode. Early pulmonary delivery systems relied on the direct instillation of aerosolized vectors, which can result in the induction of adverse reactions because vector is delivered into the lung parenchyma. More recent studies have examined the potential for using spray technologies to target aerosolized AAV vectors to the larger central airways, thereby avoiding alveolar exposure and adverse effects. Comparisons of lung deposition with nebulized delivery of aerosol and spray delivery indicate that spraying results in a more localized deposition pattern (predominantly in the proximal airways) and significantly higher deposition fractions than nebulization. These findings could lead to more efficient and targeted lung delivery of aerosolized gene vectors in the future.

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Key words: adeno-associated virus; adenovirus; cationic liposome; cystic fibrosis transmembrane conductance regulator; Escherichia coli; lipoplex; microsprayer; natural killer cells; nebulizer; neutrophil-mediated inflammatory response; Pseudomonas aeruginosa; rhesus macaque monkeys; transfection; viral protein; viral vector

Abbreviations: AAV = adeno-associated virus; Ad = adenovirus; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; rAAV = recombinant adeno-associated virus

Cystic fibrosis (CF), the most common hereditary lethal disease in the United States, is an autosomal-recessive disorder caused by mutations of the CF transmembrane conductance regulator (CFTR) gene. The genetic defect leads to disordered ion transport in the airways and the ducts of exocrine glands such as the pancreas. Defective ion transport in the airways leads to underhydrated, hyperviscous exocrine secretions and impaired mucociliary clearance. The intrapulmonary environment of CF is marked by chronic infection with a number of bacterial pathogens, most notably Pseudomonas aeruginosa, and by an exaggerated neutrophil-mediated inflammatory response to those pathogens. Products derived from infiltrating neutrophils, such as neutrophil elastase, cause airway injury and further impairment of mucociliary clearance, thus initiating a vicious cycle of infection and inflammation. The resulting airway obstruction is the major cause of early death from CF.

Gene Therapy for CF

With the identification of the CFTR gene in 1989, the theoretical possibility of correcting the CF phenotype moved closer to reality. Because patients with CF develop lung involvement after birth, CFTR gene replacement during the neonatal period prior to the onset of airway damage would, in theory, significantly decrease morbidity and mortality from CF. However, because the role of the CFTR gene in the pathologic process is incompletely understood, it is not yet clear how efficient CFTR expression will need to be in order to reverse the cycle of infection, inflammation, and obstruction that constitutes CF.

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124S History and Evolution of Aerosolized Therapeutics
The goal of developing an effective genetic therapy for CF lung disease has led to the attainment of several milestones in the larger field of gene therapy. These include the first published in vivo gene transfers with adenovirus (Ad),1,2 and with recombinant adeno-associated virus (rAAV),3 and the first phase I clinical trials using each of these vector systems.4,5 However, a number of obstacles still remain before clinically effective gene therapy will be available for the treatment of CF airway disease. Choice of vector, mode of delivery to the airways, translocation of genetic information, and expression of normalized CFTR in sufficient amounts to correct the CF phenotype in the lungs of CF patients continue to be hurdles in the development of gene therapy for CF.

**CHOICE OF VECTOR**

Several methods are currently available to transfer genetic material. In transduction, or virus-mediated gene transfer, recombinant DNA techniques are used to insert the normal copy of the needed gene into the genetic material of a virus, which then acts as a carrier or vector for gene transfer. The properties of the viral vector dictate the safety and efficacy of the gene transfer process. Transduction owes its efficiency in the transfer of genetic information to the fact that many viruses have mechanisms that enable their entry, integration, and persistence in human cells.

Transfection is another method for transferring genetic material into eukaryotic cells. It involves the transfection of bacteria such as *Escherichia coli* with DNA by chemical or physical means. Transfection may be accomplished in vitro in a wide range of cell types via cationic lipid DNA complexes known as liposomes or lipoplexes. Liposomal delivery systems generally have been less efficient for in vivo gene delivery than viral vectors, but they also have resulted in fewer inflammatory and immunologic reactions than viral vectors. Some liposomal delivery systems have shown promise for in vivo gene transfer to the airways by means of tracheal injection, aerosolization, or nasal inoculation.

The ideal vector for gene delivery would comprise the following:

- have adequate capacity to carry necessary genetic information and be undetectable by the immune system;
- be noninflammatory;
- be safe to the human host even in the setting of lung inflammation;
- be capable of whatever level of expression is needed to correct the basic CF defect;
- have a long duration of expression; and/or
- have the ability to be safely readministered (Table 1).

Therapeutic gene transfer in CF lung disease must be accomplished in vivo. Thus, the vectors require the ability to transduce cells in situ rather than undergoing ex vivo gene transfer prior to reimplantation, as can be done with hematopoietic progenitors and lymphocytes. Only a small number of vector systems with this capability have been explored to date.

Some researchers in the field argue in favor of viral vectors on the grounds that viruses have evolved and survived precisely because of their ability to enter host cell nuclei and express their genes. Others contend that the human body has developed a parallel ability to defend against viral infection. They argue that a synthetic vector would be more capable of a stealth approach.7

**Viral Vectors**

There are several theoretical concerns when employing viral vectors in the setting of CF. One is that the viral vectors themselves are exposed to the elevated levels of inflammatory mediators and their products that are present in CF airways. A second consideration is host toxicity due to the intrinsic properties of the viruses chosen as vectors.

The first vectors used for in vivo gene delivery to the lungs of animals11,2 and humans4,6 were based on group C Ads. These viruses are naturally efficient at in vivo infection of the respiratory tract, where they cause symptoms generally associated with the common cold (Fig 1).

The initial Ad vectors were adapted by deleting the immediate early genes, E1a and E1b, which are initially activated in the Ad cell life cycle. A later version of the first-generation Ad vector also deleted the early gene E3, which is required for DNA replication. The majority of early animal and human studies showed that these Ad vectors, although quite efficient for gene transfer, had dose-limiting inflammatory effects because of the relatively large amount of viral protein that was delivered to the host.4,8–11 This dose-limiting toxicity was decreased in later studies in which small volume instillations or micro-spray techniques were used.11

The immune response to the first-generation Ad vectors included the following: innate immune responses not directed at specific epitopes; cell-mediated immune responses directed against Ad protein products encoded by viral genes remaining in the viral DNA12–15; and humoral immune responses directed primarily at capsid components present on initial exposure to the recombinant virus.16

The innate immune response is related to the dose-related inflammation seen with large-volume infusions of vector. It is thought to be related to alveolar macrophages and natural killer cells, which are present in greater numbers in the distal respiratory tract regions. The problem of cell-mediated immunity has been addressed by deleting more viral coding sequences.17

A second generation of Ad vectors has been manufactured with more viral coding sequence deletions in the

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**Table 1—Characteristics of the Ideal Vector**

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<td>Adequate carrying capacity</td>
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<td>Undetectable by the immune system</td>
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<td>Noninflammatory</td>
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<tr>
<td>Safe to the human host with lung inflammation</td>
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<tr>
<td>Efficiency sufficient to correct the basic CF defect</td>
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<td>Long duration of expression and/or the ability to be safely readministered</td>
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E2a and E4 regions. These vectors have been shown to have a longer duration of activity and to elicit a lesser degree of cell-mediated immunity in some mouse strains. A more recent generation of Ad vectors (known as gutless, helper-dependent Ad vectors or high-capacity Ad vectors) has no viral genes remaining. They are less prone to destruction via cell-mediated immunity and may have somewhat enhanced duration of expression in some animal models.

Despite these changes in the Ad vectors, the problem of humoral immune responses to the Ad capsid remains. Studies in animals and humans have demonstrated that neutralizing antibodies to the Ad capsid develop following repeated administration of the vectors. In animal studies, it appears that neutralizing antibodies directed against the protein shell of the virus prevent interaction with the virus on the target cell surface or within the cell. In the latter case, antihexon antibodies may block the Ad from reaching the nucleus of target cells even after it has entered the cell. These neutralizing antibodies frequently are capable of blocking the activity of subsequent administrations. In humans, it is theoretically possible that the high levels of neutrophils and, therefore, of neutrophil elastase found in the airways of CF patients may be capable of destroying the antibodies.

While the development of neutralizing antibodies may be a problem in any viral vector system, it is likely to be circumvented most efficiently by developing a vector system in which only one administration, or widely spaced administrations, will be sufficient for gene transfer. Other possible strategies include pharmacologic immunosuppression at the time of repeat viral administration, the creation of a stealth vector with a coating that is unrecognizable by the immune system, modification of immunogenic epitopes, or possibly the induction of tolerance to Ads. This approach could theoretically entail the risk of inducing tolerance to wild-type Ads that patients might encounter.

One approach to modifying immunogenic epitopes would involve alternating vectors from one administration to the next. The rationale for this approach is that immunity against a particular viral serotype is of limited duration. A related strategy would be to use the same vector for repeated administration but to alternate the serotype, as antibodies to one serotype do not block infection by other subgroups of viruses.

Adeno-associated virus (AAV), a nonpathogenic parvovirus, has been isolated from the respiratory and GI tracts of humans. Its replication is generally dependent on coadministration with a helper virus, usually an Ad or a
herpesvirus. However, in the absence of a helper virus, a latent infection is established and may include stable integration of the AAV genome into the AAVS1 site on the chromosome. The rationale for developing AAV as a vector for gene transfer is based on mimicking this latency pathway to mediate long-term gene transfer. Because of the relatively small size of AAV, all viral genes are deleted in the production of AAV-CFTR vectors, meaning that the least amount of immunogenic material remains. This alteration, however, leaves little space for an optimal promoter sequence. The deletion of the AAV rep gene, required for replication, results in a loss of the site specificity of the integration. However, it has been shown that even with the rep deletion, AAV vectors have been capable of long-term persistence through random-site integration and episomal persistence (Fig 2).

Studies in rabbits have demonstrated locally high levels of gene transfer without adverse effects. In humans, two phase I trials have been undertaken using AAV-CFTR. Preliminary results of a nearly completed dose-escalation study including 19 subjects with mild CF lung disease have shown that AAV-CFTR administration does not induce inflammatory responses over a wide range of doses.

In a second study of AAV-CFTR administration to the paranasal sinuses of CF patients, this therapeutic complex resulted in gene transfer to the epithelium at a rate of one copy of the gene for every 10 cells. This level of transfer appeared to be stable for up to 10 weeks. This study also gave evidence of some level of electrophysiologic correction. It is not known, however, whether the desired level of CFTR messenger RNA expression is attained using this vector system. The level of messenger RNA expression is difficult to determine because of the small size of the AAV vector relative to the CFTR gene. This small size leaves no space within the vector for any vector-specific sequences that could be used in assays to distinguish vector-expressed RNA from endogenous CFTR messenger RNA and to determine whether the small endogenous AAV promoter is sufficiently active to confer the desired level of messenger RNA expression. While these first-generation AAV vectors are continuing to undergo clinical evaluation, newer versions also are being developed and evaluated. The latter include larger and more active promoters driving truncated versions of CFTR. In general, AAV vectors hold promise for gene therapy as they appear to be safe and have superior duration profiles.

In summary, it is important to view viral vectors in the context of the biological properties of the parent virus (Fig. 3). The advantages and limitations of each viral vector are directly related to those of the virus that is used. Thus, Ad causes transient infection associated with acute inflammation and so do Ad vectors. AAVs establish latency in cells without inducing inflammation, and this also is an advantage of the AAV vectors.
Cationic liposomes are lipid:DNA complexes that were developed for the transfection of eukaryotic cells in vitro. Typically, they have a cationic end, which enhances complexation with DNA and condensation into relatively small particles, and a lipophilic end, which enhances cell uptake and membrane fusion.\(^{7,20}\) Cationic liposomes are believed to form large complexes in which positively charged side groups interact with the DNA and the hydrophobic lipid portion either enhances the condensation of the DNA complex or promotes fusion with the cell membrane, or both.\(^{17}\)

Several of these liposomes have shown promise for in vivo gene transfer when administered either systemically or directly to the lung via tracheal injection, aerosolization, or nasal inoculation.\(^{30–37}\) Although liposome vectors generally have shown lower efficiency than viral vectors, they do not stimulate inflammatory and immunologic responses, which have limited the safety and efficiency of viral vectors. Studies in knockout mice\(^{32–34}\) and in humans\(^{36}\) have shown that liposome-mediated transfer of CFTR can partially correct the electrophysiologic defect. While gene transfer generally has been transient, repeat administration has not appeared to cause difficulties.

The published clinical trials\(^{39–42}\) have tested several different cationic lipids. The results of these clinical trials have indicated that gene transfer to the upper airway epithelium in humans usually is transient and inefficient but safe. The aerosol administration of Genzyme lipid 67.CFTR plasmid complexes to the lungs resulted in febrile flu-like illnesses in seven of eight subjects.

In one study, naked DNA was used as a control and resulted in significant CFTR gene transfer. Other studies have examined the use of molecular conjugates both in vitro and in animals.\(^{53–56}\) Gene transfer efficiency was demonstrated to be relatively efficient when receptors such as the polymeric Ig receptor were targeted. However, repeat dosing was inhibited by immune responses.\(^{47}\)

**Future Directions in Vector Development**

While Ad, AAV, and cationic liposome vectors are the only ones that have been subjected to clinical testing in humans with CF, others are under development. Other viral vectors that have been developed include murine leukemia retroviruses, which have been used for gene transfer in vitro and in human trials of ex vivo transfer of hematopoietic progenitors and lymphocytes.\(^{48}\) Lentiviral vectors, including those based on HIV, feline immunodeficiency virus, and equine infectious anemia virus, do not require active host cell proliferation for transduction to occur.\(^{17}\) Initial in vitro studies have been promising. However, safety concerns need to be addressed prior to the initiation of clinical trials. Other viral vectors under study include those developed from herpesviruses, DNA viral vectors based on SV40, and those based on Epstein-Barr virus, papilloma virus, and poxviruses.

Molecular conjugates are another class of vectors comprised of DNA:protein complexes designed to take advantage of receptor-mediated endocytosis to deliver DNA to cell nuclei. Although these molecular conjugates have shown in vitro efficacy in both the lung and the liver, they have not been submitted to clinical testing.

**Modes of Delivery**

A parallel challenge to the development of clinically feasible gene therapy is that of vector delivery. The development of an effective pulmonary delivery system is of paramount importance to the success of gene therapy in CF, since the products that will be used for CFTR gene therapy are difficult and expensive to manufacture. The ability to deliver the necessary dosage to the lung target site with a minimum of waste will be critical to the feasibility and success of gene therapy in CF patients.

The majority of experience in terms of vector delivery to the lungs has involved the instillation of large volumes of vector-containing fluid into the lung via the nose. However, this mode of delivery poses safety problems because of the potential for aspiration. In addition, the instillation of large volumes of fluid leads to enhanced alveolar exposure, as a result of bulk flow into the lung parenchyma. This exposure is undesirable because it may induce adverse reactions. At the same time, it is likely that alveolar epithelial cells, rather than alveolar epithelial cells, are the appropriate target for CFTR gene transfer.

Another mode of lung delivery for vector-containing fluid is by oral inhalation of aerosolized vectors. However, aerosolization of a fluid is typically achieved by means of a nebulizer, and most nebulizers have been designed to generate small particles (ie, < 5 \(\mu\)m). This is because most nebulizers have been developed to deliver drugs to treat patients with asthma, and in asthma the target region of the lungs is often the peripheral airways. Small particles enhance delivery to the peripheral airways and the alveolar region of the lung, but this is again undesirable for gene vector delivery because of the possibility of inducing adverse effects.

One way to avoid alveolar deposition is to generate an aerosol that is composed primarily of large droplets. This was the approach taken by Cipolla et al.\(^{49}\) They used a
spray delivery device that generated the Ad vector as very large particles (median diameter, 190 μm). Only 1% of the droplets were < 10 μm. Alveolar deposition was further minimized by restricting the delivered volume to < 150 μL. Delivery was achieved by means of an endoscopic washing pipe (model PW-6p; Olympus; Lake Success, NY) that could be inserted into the channel of a bronchoscope. In these experiments, the vector liquid was admixed with the radioisotope 99mTc, in order to mark where the droplets deposited in cast models of human airways. Localization of the deposited spray was determined by gamma scintigraphy and by the measurement of radioactivity exiting the terminal airways of the model. Results from these experiments indicated that vector particles generated and delivered by the spray device were localized in one to two airway generations and covered an area approximating 2 cm². These findings suggest that the delivery of the aerosolized vector by this spray device could limit deposition to specific airways and avoid alveolar exposure.

To determine the most efficient mode of delivery for future vector administration, Beck and colleagues50 have taken this approach one step further. They compared several modes of nebulizer delivery with that of spray delivery in terms of the amount of aerosol deposited in the lungs of sedated, spontaneously breathing rhesus macaque monkeys who were sitting upright. For these experiments, a saline solution aerosol was admixed with 99mTc and was generated by a nebulizer (PARI LC++; PARI Respiratory Equipment; Richmond, VA) and a compressor (Proneb; PARI Respiratory Equipment) through a mouthpiece, laryngeal mask airway, or endotracheal tube. According to the manufacturer specifications, the median diameter of particles generated by this nebulizer and compressor is 4 μm. The radiolabeled aerosol also was generated and delivered by a sprayer (MicroSprayer; Penn Century; Philadelphia, PA) that was inserted through a bronchoscope. The median diameter of particles generated by the sprayer was 22 μm. The amount of radiolabeled saline solution aerosol that deposited in the lungs (deposition fraction) was quantified from gamma camera scans of the lungs obtained immediately after each type of aerosol administration. The deposition fraction was expressed as a percentage of the starting dose.

Results from this study indicated that average lung deposition fractions in these monkeys were very low when aerosol was generated by the nebulizer and administered through the mouthpiece (0.67%), laryngeal mask airway (1.54%), or endotracheal tube (1.87%). The lung deposition fraction was significantly higher when aerosol was generated and administered by the sprayer, averaging 93%. Although extrapolating these findings to humans requires caution, they do suggest that spray delivery may have applicability to treating human infants with AAV-CFTR vectors. This is because these infants will probably require high doses of the vector for effective treatment. However, deposition fractions of aerosols that are generated by nebulizer and delivered to infants using a face-mask are similar to those reported in these experiments with rhesus macaque monkeys, averaging only about 1% of the nebulized dose.51 It may be that spray delivery would be more efficient, delivering a higher percentage of the dose to the lungs and minimizing the amount of wasted material.

Delivery of the vector by means of a spray device that is inserted into a bronchoscope may have another advantage over nebulization. In another study, Beck et al52 used the sprayer (Microsprayer; Penn Century) to deliver radiolabeled AAV2-green fluorescent protein (GFP) vector to the lungs of rhesus macaque monkeys and quantified the percentage of the aerosol that deposited in central regions of the right lung vs peripheral regions. They found that deposition was greatest in the central regions, averaging 12 to 14%, compared to only 0.23 to 7% in peripheral regions. These results suggest that spray delivery of the vector could provide a means of targeting the larger, central airways, avoiding deposition in the smaller airways and alveolar region, which is more likely with nebulizers that generate small aerosol particles. Taken together, the findings from these studies using spray technology indicate that efficient and targeted delivery of aerosolized gene vectors to the lungs may be possible in the future.

Another word of caution is required when examining the usefulness of aerosol administration of gene vectors. This is because it is important to test whether the gene vector remains intact and viable after any aerosolization process. It is possible that some nebulizers may damage the vector to a greater extent than others because of differences between devices in terms of the baffles used to break up the fluid during aerosol generation or because of the addition of heat during ultrasonic nebulization.

**Future Directions**

Great strides have been made in learning about the pathophysiology of CF lung disease, the specific barriers to CFTR gene transfer in such lungs, and the benefits and limitations of viral and synthetic vector systems. Nevertheless, additional barriers to gene therapy in CF and other disease states remain. With regard to the manufacture of vectors, host immune responses and inflammatory reactions have limited the stable persistence of the vectors at the target site, a critical factor in the correction of the underlying genetic defect. This is a particular concern with high-dose administration or repeated administration of therapy.

More data are needed regarding the role of the CFTR defect in CF so as to determine what level of corrected CFTR expression is needed to treat CF effectively. Since CF lung involvement begins early in life, further studies

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**Table 2—Findings of the National Institutes of Health Advisory Panel Regarding Gene Therapy**

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<tr>
<th>Findings of the National Institutes of Health Advisory Panel Regarding Gene Therapy</th>
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<tbody>
<tr>
<td>Gene therapy is a logical outgrowth of molecular research.</td>
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<tr>
<td>There is no definite evidence of clinical efficacy from gene transfer trials to date.</td>
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<tr>
<td>Problems remain with every aspect of gene therapy research (i.e., understanding of disease pathogenesis, vectors, preclinical animal models of disease, and clinical trial design).</td>
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<td>Gene therapy is being “oversold.”</td>
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will be needed to evaluate the optimal age for the safe initiation of therapy. Although >200 trials of gene therapies have been undertaken, no gene therapy has yet received US Food and Drug Administration approval. Table 2 summarizes the findings of an advisory panel convened by the National Institutes of Health that analyzed the reasons for this lack of approval.

REFERENCES

4 Crystal RG, McElvany NG, Rosenfeld MA, et al. Administration of an adeno-associated virus containing the human CFTR cDNA to the respiratory tract of individuals with cystic fibrosis. Nat Genet 1994; 4:42–51
43 Ferkol T, Kaetzel CS, Davis PB. Gene transfer into respiratory epithelial cells by targeting the polymeric immunoglobulin receptor. J Clin Invest 1993; 92:2394–2400
52 Beck SE, Laube BL, Barberena CT, et al. A threshold regional dose of AAV vector is necessary to detect transduction in the lower airways of rhesus macaques [abstract]. Pediatr Pulmonol 2000; (suppl):A228