Published in final edited form as:

Hum Gene Ther. 2006 April ; 17(4): 440–447. doi:10.1089/hum.2006.17.440.

Prevalence of Neutralizing Antibodies against Adeno-Associated Virus (AAV) Types 2, 5, and 6 in Cystic Fibrosis and Normal Populations: Implications for Gene Therapy using AAV Vectors

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Abstract

Adeno-associated virus (AAV) vectors are promising candidates for gene therapy directed to the lungs, in particular for treatment of cystic fibrosis (CF). In animal models of lung gene transfer, neutralizing antibodies in serum made in response to vector exposure have been associated with a partial to complete block to repeat transduction by vectors with the same capsid type, thus transduction by AAV vectors might be inefficient in humans previously exposed to the same AAV type. AAV type 2 (AAV2) has been used in clinical trials of lung gene transfer, but AAV5 and AAV6 have been shown to mediate more efficient transduction in rodent lungs and in cultured human airway epithelia compared to that of AAV2. Here we have measured neutralizing antibodies against AAV type 2, 5, and 6 vectors in serum from children and adults with CF, and from normal adults. About 30% of adults were seropositive for AAV2, 20–30% were seropositive for AAV6, and 10–20% were seropositive for AAV5. CF children were seropositive for AAV types 2, 5, or 6 at rates of 4–15%. All individuals seropositive for AAV6 were also seropositive for AAV2, and the AAV6 titer was low compared to the AAV2 titer. AAV5-positive sera were lower both in titers and rates than those seen for AAV6. The results indicate that AAV type 2, 5 or 6 exposure is low in CF and control populations and even lower in CF children.

INTRODUCTION

Cystic fibrosis (CF) is the most common lethal inherited disease in the white population. CF is an autosomal recessive disorder caused by mutations in a single gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The CFTR is a member of the ATP-binding cassette family of transporters and in part functions as a chloride channel. Abnormal CFTR function results in alterations of airway surface liquid and mucociliary clearance that are associated with chronic endobronchial bacterial infection and inflammation leading to progressive obstructive lung disease (Gibson *et al.*, 2003).

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Introduction of a normal CFTR gene into a CF airway epithelial cell line has been shown to restore normal chloride transport (Drumm *et al.*, 1990), which in turn could prevent the onset of repeated bacterial infections leading to lung pathology. Although the normal CFTR protein has been localized to both the apical surface of the airway epithelium (Yankaskas *et al.*, 1993) and to the submucosal glands beneath the epithelium (Engelhardt *et al.*, 1992), the airway is the site of microbial infection associated with mortality. This clinical manifestation provides the rationale for targeting the airway epithelium for CF gene therapy.

Among the many gene transfer systems being investigated for in vivo delivery, viral vectors based on adeno-associated virus (AAV) show great promise. AAV vectors can promote persistent gene expression in cultured cells and in dividing and nondividing cells in multiple somatic tissues of animals (Kessler *et al.*, 1996; Herzog *et al.*, 1997, 1999; Koeberl *et al.*, 1997, 1999; Snyder *et al.*, 1997). The ability to transduce nondividing cells (Russell *et al.*, 1994) is an important feature of AAV vectors for gene transfer to the airway epithelium because of its low rate of proliferation (Ayers and Jeffery, 1988). Wild-type AAV has not been associated with human disease, thus vectors based on AAV are expected to be inherently safe.

Vectors bearing AAV type 2 (AAV2) capsid proteins can transduce multiple cell types in the lungs of animals, but at low to modest transduction rates (Fisher *et al.*, 1997; Halbert *et al.*, 1997; Allen *et al.*, 2000). The low transduction rate seen with AAV2 vectors in the airway epithelium is most likely due to the limited binding of the AAV2 capsid to the apical surface of the airway epithelium, which has a low abundance of the putative AAV2 receptor, heparan sulfate proteoglycan (HSP) (Duan *et al.*, 1998; Summerford and Samulski, 1998). In contrast, AAV vectors bearing capsid proteins from AAV types 5 or 6 show high transduction rates in rodent lungs and in cultured human epithelia, with transduction rates achieved by AAV6 in the range estimated to be sufficient for treating cystic fibrosis (Zabner *et al.*, 2000; Halbert *et al.*, 2001). It is uncertain whether these results will be predictive of results in humans.

Immune responses can limit viral gene transfer and persistence. In readministration studies using AAV2 vectors, little to no new transduction events were detected after the second administration of an AAV2 vector in rabbit or mouse lung, in mouse skeletal muscle, or in mouse liver, and poor transduction in the second administration was associated with the presence of neutralizing antibodies to AAV2 capsid proteins resulting from the first vector administration (Fisher *et al.*, 1997; Halbert *et al.*, 1997, 1998; Manning *et al.*, 1998; Xiao *et al.*, 1999). This suggests that successful gene delivery using AAV vectors might be difficult in humans previously exposed to the same AAV type. Previous use of AAV vectors in CF clinical trials have all employed an AAV2 vector encoding CFTR. Repeat delivery of this vector was associated with a rise in AAV2-neutralizing antibodies, as anticipated (Moss *et al.*, 2004). However, no gene expression was observed in the first or subsequent vector administrations, thus it is impossible to know whether transduction was inhibited in later vector administrations.

In contrast to the impaired transduction by AAV vectors found with repeat vector administration to mice and rabbits, others have claimed that repeat transduction is possible

in immunocompetent rhesus macaques (Fischer *et al.*, 2003). In this study, two doses of an AAV2-CFTR vector were followed by administration of an AAV2 vector expressing green fluorescent protein (GFP). Although fluorescence suggestive of GFP expression was found by microscopy and GFP protein expression was detected by Western analysis, there was little correlation with the level of messenger RNA, which when present, was not within the limits of detection for quantitative analysis. The rate of transduction of lung cells was not quantitated, and GFP expression was not examined in naive animals that received only the GFP vector, so it is impossible to determine whether GFP expression was reduced in preimmunized animals in comparison to naive animals. Therefore, although this study indicates that an AAV2 vector can transduce cells in the rhesus lung after previous administration of AAV2 vectors, prior studies in mice and rabbits and general principles of immunology strongly support the conclusion that immune responses resulting from AAV infection will limit transduction by AAV vectors bearing the same capsid proteins.

Here we have tested for the presence of neutralizing antibodies to AAV types 2, 5, and 6 in CF and normal human subjects. Although antibodies that recognize a virus can be easily assayed by enzyme-linked immunosorbent assay, we measured neutralizing antibodies because antibodies that interfere with virus–cell interaction are more likely to determine gene therapy outcomes. We were particularly interested in determining the age-related prevalence of antibodies. Here we present data from 89 CF children (under 18 years of age), 40 CF adults, and 37 normal adults.

MATERIALS AND METHODS

Cell culture

Human embryonic kidney 293 cells (Graham *et al.*, 1977) and human HTX cells (an approximately diploid subclone of HT-1080 fibrosarcoma cells [CCL-121; American Type Culture Collection, Manassas, VA]) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS).

AAV vector production and characterization

The AAV2-based vector ARAP4 (Allen *et al.*, 2000) contains an expression cassette consisting of the Rous sarcoma virus (RSV) promoter and enhancer sequences, a human placental alkaline phosphatase (AP) cDNA, and a simian virus 40 (SV40) polyadenylation signal. The expression cassette is flanked by AAV2 terminal repeats. ARAP4 vectors with AAV2, AAV5, or AAV6 capsid proteins were generated by cotransfection of HEK 293 cells seeded the previous day at 4×10^6 cells per 10-cm dish with four plasmids: the vector plasmid pARAP4, a plasmid encoding the AAV2 Rep protein (pMTrep2) (Allen *et al.*, 2000), a plasmid encoding Cap proteins from either AAV2 (pCMVcap2) (Allen *et al.*, 2000), AAV5 (pAAV5Rep-Cap) (Zabner *et al.*, 2000), or AAV6 (pCMVcap6) (Halbert *et al.*, 2001), and a helper plasmid encoding the adenovirus type 5 E4orf6 protein (pCMVE4orf6) (Allen *et al.*, 2000).

Concentration and purification of the pseudotyped AAV vectors (same genome but different capsid proteins) AAV2-AP, AAV5-AP, and AAV6-AP were done as previously described

(Halbert and Miller, 2004). Briefly, clarified crude cell lysates obtained 3 days after transfection were concentrated by centrifugation through a sucrose cushion, followed by density banding in cesium chloride, and dialysis in Ringer's saline solution. The titers of the AAV vector stocks were determined using HTX cells as targets for transduction as previously described (Halbert *et al.*, 2001). Southern analysis was done to determine the number of vector genomes (vg) in the vector preparations as described (Halbert *et al.*, 1997), and vector stocks typically contained 10^{11} to 10^{12} vg/ml.

Human subjects and collection of sera

The study was conducted after receiving Institutional Review Board approval for the research. Informed consent, and assent if applicable, were obtained for all subjects. CF subjects were recruited from the Cystic Fibrosis Research Center at Children's Hospital and Regional Medical Center (Seattle, WA), the University of Washington Medical Center (Seattle, WA), and Children's Hospital (Denver, CO). Control subjects were recruited from among non-CF adults in the Seattle community. Study subjects were recruited between May 1999 and September 2002. Microbiology results for the most recent respiratory culture were recorded at the study visit if performed within 1 year of the visit. For place of residence, data were categorized as to whether or not the subject reported ever having lived outside of the Pacific Coast states (Washington, Oregon, California, Alaska, Hawaii), that is, in other parts of the United States or abroad. For travel history, data were categorized as to whether or not the subject reported every having traveled outside the United States.

A single blood draw was performed to obtain serum for antibody assays. Blood was clotted at 4°C overnight and the serum was collected after centrifugation of clotted blood in a clinical table-top centrifuge. Serum samples were stored below -70°C until analysis. Before performing neutralization assays, serum samples were incubated at 56°C for 45 min to inactivate complement, and were then kept at 4°C.

Virus neutralization assay

To screen for virus-neutralizing activity in human serum samples, 10^8 vg of AAV2-AP, AAV5-AP, or AAV6-AP (in 100 μ l of DMEM containing 2% FBS) was mixed with 100 μ l of serum diluted in the same medium to achieve final serum dilutions of 1:20, 1:200, and 1:1000, or were incubated without serum in the same volume as a control. Dilutions of animal serum less than 1:20 sometimes exhibit nonspecific inhibition of vector transduction, so we did not examine the effects of human serum diluted less than 1:20. One hour after mixing the vectors and serum samples, 80, 10, or 2% of each mixture was added to HTX cells seeded the previous day at 10^5 cells per well ($d = 3.4$ cm) of 6-well plates. In parallel, amounts of vector (incubated in the same volume but without human serum) were added to cells for vector control standards. The cells were grown for an additional 2–3 days, fixed and stained for AP expression, and AP-positive foci were counted to quantitate the transduction rate. Serum samples were considered to have neutralizing activity if the 1:20 serum dilution inhibited vector transduction by at least 50%. The serum was further categorized as having low, medium, or high titer if a 50% or greater reduction in vector transduction was achieved with the 1:20, 1:200, or 1:1000 dilutions, respectively. A second assay was done on the positive sera to determine the neutralizing titer. Serial 2-fold dilutions starting at 1:20,

1:200, or 1:1000 were made of the serum, depending on the category of inhibition determined by the first assay. The reciprocal of the highest dilution of serum that inhibited AAV transduction (AP-positive focus-forming units [FFU]) by 50% or more compared with untreated vector was defined as the neutralizing titer.

Statistical analysis

Data were summarized as graphical displays and descriptive statistics (i.e., means and standard deviations for continuous variables, and counts and proportions for categorical variables). χ^2 tests were used to test differences in proportions between patient subgroups and to evaluate potential associations between selected risk factors and the presence of neutralizing antibodies; where indicated, a logistic regression model was used for covariate adjustment when evaluating potential associations. Results were not adjusted for multiple comparisons.

RESULTS

Demographics

Serum samples were obtained from 166 subjects (129 CF and 37 non-CF subjects). Demographic data were obtained from all subjects and included genotype for CF subjects (Table 1). The majority of the subjects were white and the majority of CF subjects were F508 homozygous. The gender split was close to 50% in all groups.

Virus neutralization assay

To compare the prevalence and magnitude of neutralizing antibodies in serum against AAV vectors having capsid proteins from AAV types 2, 5, or 6, we measured neutralization of AAV vectors that carried the same AAV2-based vector genome, were made using the same AAV2 Rep proteins, and differed only in their capsid proteins. The vectors all encoded AP, which was used as a histologic marker for transduction. To allow comparison of results between vectors containing the different capsids we used the same amount of virus in the neutralization assays, as measured by quantitation of vector genomes (vg), even though each vector exhibited a different transduction rate on HTX target cells (100, 5×10^4 , and 2×10^4 vg per AP-positive FFU for AAV types 2, 5, and 6, respectively). In addition, it was important that the assay be done under conditions of antibody excess to maximize the sensitivity of the assay and to allow comparison between the different vectors. To examine this issue, we performed neutralization assays using different amounts of each vector to determine the effect of vector amount on measurement of antibody titer (Fig. 1). Use of 10^9 vg clearly reduced the measured antibody titer for all three vectors, indicating that the antibodies were not in excess when using this amount of vector. Use of 10^8 or 10^7 vg resulted in measured titers that varied by less than 2-fold, indicating that these conditions approximated a condition of antibody excess. Because of the low infectivity of the AAV type 5 and 6 vectors, we used 10^8 vg of the AAV type 2, 5, and 6 vectors in subsequent assays to allow accurate and comparable determinations of vector titer for all vectors.

Prevalence of neutralizing antibodies to AAV2, AAV5, and AAV6 in CF and normal populations

Four groups of human subjects were analyzed for the prevalence of neutralizing antibodies against AAV types 2, 5, and 6 (Fig. 2). Sera were judged positive for neutralizing antibodies when a 1:20 dilution of serum inhibited vector transduction by 50% or more. In general, CF children had a lower prevalence of neutralizing antibodies than did adults. The prevalence of neutralizing antibodies in sera from CF adults and age-matched normal cohorts was similar.

Statistical comparisons of prevalence of neutralizing antibody activity between subgroups defined by age and/or disease status were performed. There were no significant differences in prevalence of antibodies between CF adults and non-CF adults when proportions were compared by χ^2 test ($p = 0.83$, $p = 0.45$, and $p = 0.47$ for AAV2, AAV5, and AAV6, respectively). A reduced prevalence of neutralizing antibodies was noted when children with CF (age <18 years) were compared with adults with CF (age ≥ 18 years) as shown in Table 2.

Coprevalence of neutralizing antibodies

The coprevalence of neutralizing antibodies to AAV types 2, 5, and 6 is shown in Table 3. Of 48 CF children from ages 3 months through 10 years of age, 5 were found to have activity to AAV2, and of these 2 were also positive against AAV5, and 3 against AAV6. In 41 CF children from 11 to 17 years of age, 6 were positive for AAV2 and of these 3 were also positive for AAV5, and 5 for AAV6. One monospecific serum to AAV5 was seen in an 11-year-old. Thus, 8 of 12 CF children having positive neutralizing activity (66%) had sera that neutralized more than one of the serotypes tested.

In 40 CF adults, 11 were seropositive for AAV2, and of these 8 were also positive for AAV5 and 9 for AAV6. In 37 non-CF adults, 11 were seropositive for AAV2, and of these 5 were positive for AAV5 and all 11 were positive for AAV6. Thus, 20 of 22 positive sera (91%) from CF and non-CF adults had co-occurrence of neutralizing activity to another AAV type.

Titer of neutralizing antibodies

The magnitude of the neutralizing activity to AAV types 2, 5, and 6 was measured by determining the neutralizing titer, defined as the inverse of the highest dilution that still inhibited vector transduction by 50% or more. The observed titer for each subject in whom neutralizing antibody activity was detected against any of the AAV types tested is shown in Table 4. Results were sorted by study group and age. The highest titer was seen against AAV2 in one 4-year-old CF child who had a titer of 1:12,800. AAV2 titers were higher than those to AAV5 and AAV6. All AAV6-positive sera were positive for AAV2 and the AAV6 titer was always lower than the corresponding AAV2 titer. Nineteen of 20 sera positive for AAV5 were also positive for AAV2 and AAV6, and the AAV5 titer was lower than those of AAV2 and AAV6. The AAV5-monospecific serum showed a low neutralization titer of 40.

Examination of risk factors

Among CF subjects only, data were examined for potential associations between the presence of neutralizing antibodies and the presence of selected clinical and demographic

factors (Table 5). Travel outside of the United States or residence outside of the Pacific Coast states was not associated with a higher prevalence of neutralizing antibodies. The coprevalence of *Pseudomonas aeruginosa* (Pa) and *Staphylococcus aureus* (Sa) was also examined. Almost all of the 103 subjects from whom cultures were available within a 1-year time period had one or both of these CF pathogens present (45 with Pa only, 22 with Sa only, 31 with both Pa and Sa, and 5 with neither pathogen). There was no evidence of an association between the presence of either pathogen and the presence of antibodies to AAV2, AAV5, or AAV6 when examined in a logistic regression model that adjusted for presence of the other pathogen.

DISCUSSION

We have found that the majority of CF and normal adults were negative for neutralizing activity against virions containing capsid proteins from AAV types 2, 5, and 6. Approximately 10% of CF children and 30% of adults were seropositive for AAV2. Previous estimates of AAV2 seropositivity have been higher (30% in children and 60% in adults) (Blacklow *et al.*, 1968a,b, 1971; Parks *et al.*, 1970). These differences may be due to differences in methods used (complement fixation, immunofluorescence, and enzyme-linked immunosorbent assay versus the neutralization assay used here). In addition, the earlier work used wild-type adenovirus as helper and could have been compromised by existing neutralizing activity to adenovirus that is known to be high in patient serum. More recent results show that 30% of the adult population have neutralizing activity to AAV2 (Chirmule *et al.*, 1999; Xiao *et al.*, 1999), consistent with our results.

No monospecific serum was detected against AAV6 capsid. All AAV6-positive sera were also AAV2 positive. One monospecific serum to AAV5 was seen in an 11-year-old. The other 18 AAV5-positive sera were also AAV2 positive. In all sera having neutralizing activity to more than one AAV type, the AAV2 titer was much higher. One hypothesis for these results is that most AAV5- and all AAV6-neutralizing activities were due to cross-reacting antibodies generated by exposure to AAV2, and that AAV5 and AAV6 viruses are rare in human populations. If this is the case, then the presence of cross-reacting antibodies in human serum is different from that found in animal studies of AAV vector readministration. Those studies showed that serum antibodies formed after a single AAV2 vector administration did not neutralize or prevent transduction by AAV6 or AAV1 vectors, but did prevent AAV2 transduction in a second exposure (Manning *et al.*, 1998; Xiao *et al.*, 1999; Halbert *et al.*, 2000). The antibodies to AAV2 obtained in rabbits and rodents also did not neutralize AAV5 vectors (data not shown). The seropositivity seen to AAV5 and AAV6 suggests that humans can generate cross-reacting antibodies to other AAV types after exposure to wild-type AAV2. One would expect cross-neutralizing activity to be lower than that against AAV2, and indeed this is the case for our data.

Alternatively, the co-occurrence of neutralizing antibodies to AAV2, AAV5, and AAV6 may be the result of multiple or coinfections within the same individual. AAV2 may be more immunogenic in stimulating a robust neutralizing response than AAV types 5 and 6. Interestingly, transduction by AAV6 is possible even in animals previously exposed to an AAV6 vector (50% of the level found in naive animals) (Halbert *et al.*, 2000). The

neutralizing titers seen in those animal studies were low (<100), and correlated with the ability to readminister an AAV6 vector. This suggests that AAV6 is less immunogenic than AAV2 in rodents, and this may be the case in other species. Whatever the reason, these antibodies can neutralize AAV vectors and may affect the outcome of gene therapy using these AAV types. Alternatively, the low neutralizing titers against AAV5 and AAV6 may be insufficient to inhibit lung gene transfer.

The prevalence of neutralizing antibodies against AAV types 2, 5, and 6 in children was of particular interest because these individuals have fewer clinical manifestations of lung pathology and therefore would be more responsive to gene therapy intervention. Of note, none of the 12 CF children aged 0–3 years had detectable neutralizing antibodies to any AAV type, and none of the 7- to 10-year-olds had antibodies to AAV5 or AAV6. In fact, antibodies to AAV2 were not consistently detected in each age group until age 12 years. Although the numbers of samples are small at each age group, the data are consistent with the report that children are initially exposed to AAV during the preschool age and at a low rate thereafter (Parks *et al.*, 1970). The low incidence of AAV-neutralizing activity throughout childhood is encouraging as it provides a wide window of opportunity for gene therapy with AAV vectors made using several capsid types.

Here we show that the majority of CF children and adults studied were negative for neutralizing activity against vectors made with capsid proteins from AAV types 2, 5, and 6. The prevalence and strength of the immune responses indicate that vectors made with AAV5 and AAV6 capsids will be most useful for avoiding preexisting immunity during lung gene therapy. It remains to be seen whether cross-reacting and non-cross-reacting antibodies generated by administration of one vector type in humans will allow successful transduction by vectors with the same or different capsid proteins.

Acknowledgments

The authors thank John Alfano and Siu-Ling Lam for excellent technical assistance; the CF Centers at Children's Hospital and University of Washington Hospital for patient recruitment, serum collection, and data compilation; and Frank Accurso and Marci Sontag from The Children's Hospital in Denver and the University of Colorado School of Medicine for providing two serum samples from children who were included in this study. This work was supported by grants DK47754 and HL66947 from the National Institutes of Health and by grants from the Cystic Fibrosis Foundation.

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OVERVIEW SUMMARY

Cystic fibrosis (CF) is one of the most common human genetic diseases. The disease is caused by defects in the cystic fibrosis transmembrane conductance regulator (CFTR), and the majority of the morbidity and mortality could be alleviated by expression of normal CFTR protein in cells of the lung. AAV vectors, especially those made with capsid proteins from AAV types 5 or 6, can provide high rates of transduction in lungs of animals and thus may be useful for CF gene therapy. However, preexisting immunity to AAV resulting from prior exposure may limit such therapy. Here we show that more than 70% of CF adults and more than 85% of CF children lack serum neutralizing antibodies against AAV types 2, 5, or 6. Furthermore, 95% of CF children aged 0–10 years lacked serum neutralizing antibodies against AAV types 5 or 6. These results indicate that preexisting immunity will not limit AAV vector-mediated gene therapy for the majority of CF individuals.

FIG. 1.

Effect of vector concentration on measurement of serum neutralization titer. One human serum sample with moderate titer against AAV2 and AAV6 was used for the AAV2 and AAV6 analyses, and another human serum sample with a moderate titer against AAV5 was used for the AAV5 analysis. Serial 2-fold dilutions of serum in 100 μ l of medium (DMEM with 2% FBS), or 100 μ l of medium alone, were added to 100 μ l of medium containing 10⁷ vg (diamonds), 10⁸ vg (squares), or 10⁹ vg (triangles) of AAV type 2, 5, or 6 vector, and the mixtures were incubated at 37°C for 1 hr. Next, the vector and serum mixtures were added to HTX cells to measure vector titer and neutralization as described in Materials and Methods.

FIG. 2.

Prevalence of neutralizing antibodies in sera. Sera were judged positive if a 1:20 dilution of serum inhibited vector transduction by at least 50%.

Table 1

Demographics of Study Population

Characteristic	CF children ages 0–10 yr (N=48)	CF children ages 11–17 yr (N=41)	CF adults ages 18 yr (N = 40)	Non-CF adults ages 18 yr (N = 37)
Mean age, yr (SD)	5.7 (3.1)	14.2 (2.1)	28.5 (7.8)	29.6 (7.1)
[min, max]	[0.3, 10.9]	[11.0, 17.7]	[18.0, 47.2]	[18.9, 46.7]
Sex				
Females	25 (52%)	19 (46%)	18 (45%)	17 (46%)
Males	23 (48%)	22 (54%)	22 (55%)	20 (54%)
Race				
White	45 (94%)	41 (100%)	40 (100%)	31 (84%)
Other	3 (6%)			6 (16%)
CF Genotype				
F508/ F508	30 (63%)	30 (73%)	20 (50%)	---
F508/other	15 (31%)	9 (22%)	17 (43%)	---
Other/other	3 (6%)	2 (5%)	3 (7%)	---

Table 2

Comparisons of Antibody Prevalence between Children and Adults with CF

Vector	Seropositive CF children (N = 89)	Seropositive CF adults (N = 40)	P-value for difference (χ^2 test)
AAV2	11 (12.4%)	11 (27.5%)	0.03
AAV5	6 (6.7%)	8 (20.0%)	0.03
AAV6	8 (9.0%)	9 (22.5%)	0.04

Table 3

Coprevalence of Neutralizing Antibodies to AAV Types 2, 5, and 6

Seropositivity	Number of subjects with indicated seropositivity in:			
	CF children ages 0–10 yr (N = 48)	CF children ages 11–17 yr (N = 41)	CF adults ages 18 yr (N = 40)	Non-CF adults ages 18 yr (N = 37)
None	43 (89.6%)	34 (82.9%)	29 (72.5%)	26 (70.3%)
AAV2	2 (4.2%)	1 (2.4%)	2 (5.0%)	---
AAV5	---	1 (2.4%)	---	---
AAV6	---	---	---	---
AAV2, AAV5	---	---	---	---
AAV2, AAV6	1 (2.1%)	2 (4.9%)	1 (2.5%)	6 (16.2%)
AAV5, AAV6	---	---	---	---
AAV2, AAV5, AAV6	2 (4.2%)	3 (7.3%)	8 (20.0%)	5 (13.5%)

Table 4

Neutralizing Antibody Titers to AAV Types 2, 5, and 6

Group	Age	Antibody titer		
		AAV2	AAV5	AAV6
CF, 0–10 yr	4.1	80	<20	<20
	4.4	12,800	1,280	6,400
	5.9	6,400	20	320
	6.0	640	<20	320
	8.0	320	<20	<20
CF, 11–17 yr	11.7	40	<20	<20
	11.9	<20	80	<20
	12.2	200	<20	80
	14.9	3,200	<20	320
	15.4	2,000	160	200
	16.1	200	80	160
	17.6	1,600	80	160
	18.2	6,400	20	160
CF, 18 yr	18.8	5,120	80	320
	19.5	2,560	40	320
	19.9	2,000	80	160
	21.3	2,560	40	160
	24.7	2,560	80	640
	26.6	80	<20	<20
	28.1	800	40	80
	29.1	1,600	160	320
	36.1	80	<20	<20
	44.3	1,600	<20	160
Non-CF, 18 yr	18.9	2,560	<20	320
	22.3	640	40	160
	23.0	16,000	160	800
	23.6	2,000	80	80
	27.8	1,280	80	80
	30.3	16,000	<20	80
	30.5	8,000	<20	1,600
	31.2	200	<20	40
	31.7	160	<20	80
	36.3	2,560	<20	80
	46.1	32,000	160	3,200

Table 5

Associations with Presence of AAV Neutralizing Antibodies in CF Subjects

Characteristic	Prevalence *	Association with neutralizing antibodies to:		
		AAV2	AAV5	AAV6
<i>P. aeruginosa</i> infection	Negative, N=27	8 (29.6%)	4 (14.8%)	6 (22.2%)
	Positive, N=76	11 (14.5%)	9 (11.8%)	9 (11.8%)
		$p = 0.08$	$p = 0.69$	$p = 0.19$
<i>S. aureus</i> infection	Negative, N=50	6 (12.0%)	6 (12.0%)	5 (10.0%)
	Positive, N=53	13 (24.5%)	7 (13.2%)	10 (18.9%)
		$p = 0.10$	$p = 0.85$	$p = 0.20$
Have lived outside of Pacific Coast states	No, N=90	16 (17.8%)	10 (11.1%)	13 (14.4%)
	Yes, N=30	6 (20.0%)	4 (13.3%)	4 (13.3%)
		$p = 0.79$	$p = 0.74$	$p = 0.88$
Have traveled outside of USA	No, N=53	10 (18.9%)	7 (13.2%)	8 (15.1%)
	Yes, N=68	12 (17.7%)	7 (10.3%)	9 (13.2%)
		$p = 0.86$	$p = 0.62$	$p = 0.77$

* Although there were 129 subjects with CF, information on bacterial culture status, residence history, and/or travel history was missing for some subjects.