Adeno-Associated Virus Antibody Profiles in Newborns, Children, and Adolescents

Roberto Calcedo, Hiroki Morizono, Lili Wang, Robert McCarter, Jianping He, David Jones, Mark L. Batshaw, and James M. Wilson

Gene Therapy Program, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104; Center for Genetic Medicine Research, Children’s Research Institute, Children’s National Medical Center, Washington, DC 20010; and Center for Clinical and Community Research, Children’s Research Institute, Children’s National Medical Center, Washington, DC 20010

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Neutralizing antibodies (NAb) to an adeno-associated virus (AAV) vector due to previous natural infection with wild-type AAV can significantly limit gene transfer. NAb titers to AAV serotype 2 (AAV2) and AAV8 in human subjects (0 to 18 years) were studied. NAb prevalence is moderate at birth, decreases markedly from 7 to 11 months, and then progressively increases through childhood and adolescence.

Adeno-associated viruses (AAVs) are replication-defective members of the family Parvoviridae that have been widely used as vectors for gene therapy. Vectors based on AAV serotype 2 (AAV2) have been evaluated in both animal models and humans. The discovery of new serotypes, including AAV8 (6), has provided alternative vectors with substantial advantages over AAV2 in terms of gene transfer efficiency (4, 11, 13, 18), lower prevalence of neutralizing antibodies (NAb) in the human population (1, 3), and less propensity to activate T cells for capsid proteins (14, 17).

It has been shown that preexisting NAb to the viral vector limits effective gene transfer in a way that is influenced by the route of administration and organ targeted. Several studies have shown that even low levels of AAV NAb can reduce gene transfer into the liver following intravascular delivery (7, 16) in the context of potential treatments of several genetic disorders, including hemophilia B and ornithine transcarbamylase deficiency.

The goal of this study was to evaluate the prevalence of NAb to AAV2 and AAV8 in plasma from newborns, children, and adolescents to determine the ideal age interval for gene therapy intervention, which would be when the prevalence of AAV NAb is the lowest.

Plasma samples from 752 anonymous human subjects of different age groups (Table 1) were obtained from the Division of Laboratory Medicine at Children’s National Medical Center (Washington, DC). Samples were heat inactivated at 56°C for 30 min and analyzed for NAb to AAV2 or AAV8 by an in vitro transduction inhibition assay (3). NAb titers were determined for each sample, and data were recorded as counts of positive responses among totals evaluated by vector, age, and dilution and used to estimate the prevalence of vector transduction inhibition at plasma dilutions of ≥1:5, ≥1:10, ≥1:20, and ≥1:40 (Fig. 1). The NAb titer was reported as the highest plasma dilution that inhibited AAV transduction of Huh7 cells by 50% or more compared with that for the naive serum control. The limit of detection of the assay was 1:5. Stratified contingency table analyses and negative binomial regression models in the Statat 11 software program (12), appropriate for count-type data, were used to evaluate the impact of age and AAV serotype on the prevalence of seropositivity based on an AAV NAb titer equal to or greater than 1:20 (Table 1 and Table 2).

TABLE 1. Average prevalence of NAb (titer of ≥1:20) by age in anonymous serum samples from Children’s National Medical Center

| Group            | Age (yr) | No. of samples: | % prevalence | Relative prevalence | 95% confidence interval | P value |
|------------------|----------|-----------------|--------------|---------------------|-------------------------|---------|
|                  |          | Tested Positive |              |                     |                         |         |
| Infantsa         | <1       | 175             | 31           | 15                  |                         |         |
| Toddlers         | 1–<3     | 83              | 13           | 13.5                | 0.9                     | 0.49, 1.64 | 0.72   |
| Children Adolescents | 3–18    | 350             | 96           | 21.5                | 1.43                    | 0.99, 2.07 | 0.052  |

* Corresponding author. Mailing address: Gene Therapy Program, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104. Phone: (215) 898-0226. Fax: (215) 494-5444. E-mail: wilsonjm@mail.med.upenn.edu.
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had plasma dilution titers of $\geq 1:40$ for AAV2 and 13% for AAV8. Prevalence of NAb to both AAV serotypes declined significantly after birth, reaching a nadir in the 7- to 11-month group, probably due to a drop in maternal antibody levels. These results are consistent with our statistical model based on the negative binomial that indicated a rapid decline in NAb prevalence during the first 6 months of life followed by a gradual increase with age thereafter, using a NAb titer of 1:20 (%) by AAV vector DNA to the spleen (17a). Our data suggest that 70% and 82% of newborns have titers below 1:20 (Fig. 1C) and would be suitable subjects for systemic delivery of AAV2 or AAV8, respectively. This percentage would increase to 97% for AAV2 and 100% for AAV8 if genetic intervention is delayed to 7 to 11 months of age.

In summary, our data indicate that the best age for an early gene therapy intervention with an AAV vector would be between 7 and 11 months of age and that after 3 years of age AAV8 would be a better delivery vector than AAV2 due to its lower NAb prevalence. AAV-mediated gene therapy on patients of any age clearly will require careful screening for preexisting NAb due to the wide range of seroprevalences observed in the study. Our data also suggest that natural infections with AAV occur soon after the infant loses humoral protection due to passive transfer and remain stable until adolescence, when there is an apparent increase in infections.

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In these and subsequent statistical analyses. Table 1 shows the average prevalence was 15% in infants (ages < 1 year), 14% in toddlers (ages 1 to <3 years), and 21% in older children (ages 3 to 18 years), the latter increase from infancy nearly reaching statistical significance ($P = 0.052$). Table 2 compares the average NAb prevalence across all ages for AAV2 (22%) and AAV8 (15%) and indicates that this difference achieves statistical significance ($P = 0.025$). Previous studies indicated the potential intrauterine transmission of maternal AAV into the fetus due the high susceptibility to infection of the trophoblast by AAV (2, 10) and the possible transmission of AAV during vaginal delivery (5, 15). Although our serological analysis does not indicate a persistent humoral immune response to AAV after birth, as would be expected if the newborns were infected at birth, it indicates an AAV infection after 1 year of age, with a peak at 3 years of age. This serologic pattern closely follows that of the adenovirus as described previously (9) and is consistent with the acquisition of AAV as a consequence of adenovirus infection.

Recent studies in monkeys have shown that very low levels of preexisting NAb to AAV8 can abrogate AAV8-mediated liver transduction (8, 16). In studies of liver-directed gene therapy, we have shown that an AAV8 NAb titer of 1:20 is sufficient to reduce transduction considerably and to redirect vector DNA to the spleen (17a). Our data suggest that 70% and 82% of newborns have titers below 1:20 (Fig. 1C) and would be suitable subjects for systemic delivery of AAV2 or AAV8, respectively. This percentage would increase to 97% for AAV2 and 100% for AAV8 if genetic intervention is delayed to 7 to 11 months of age.

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