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# Age Dependence of Adenovirus-Specific Neutralizing Antibody Titers in Individuals from Sub-Saharan Africa

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**We assessed neutralizing antibody titers to adenovirus serotype 5 (Ad5) and six rare adenovirus serotypes, serotypes 11, 35, 50, 26, 48, and 49, in pediatric populations in sub-Saharan Africa. We observed a clear age dependence of Ad5-specific neutralizing antibody titers. These data will help to guide the development of Ad vector-based vaccines for human immunodeficiency virus type 1 and other pathogens.**

Replication-incompetent adenovirus serotype 5 (Ad5) vector-based vaccines for human immunodeficiency virus type 1 (HIV-1) have proven highly immunogenic in preclinical studies and are being advanced into large-scale clinical trials. A potential limitation of this approach, however, is that the majority of individuals, particularly in the developing world, have high levels of preexisting anti-Ad5 immunity that may limit the immunogenicity of these vaccine vectors (1, 6, 8, 12, 13). To overcome this problem, our laboratories and others have developed Ad vectors that circumvent anti-Ad5 immunity. These vectors include rare human serotype Ad vectors (5, 7, 10, 13), capsid chimeric Ad vectors (9), and nonhuman primate Ad vectors (3, 4).

There are 51 known human Ad serotypes that are divided into species A to F. Ad5 from species C has been shown to be particularly common in adults in the developing world (6, 8, 12). In contrast, several Ads from species B and D were found to be rare in a seroprevalence study conducted in a Belgian population (13). Ad seroprevalence studies to date, however, have been limited in two important ways. First, head-to-head studies of multiple Ad serotypes from species B and D in the developing world have not been reported previously. Second, Ad seroprevalence studies have only been conducted with adults, despite the fact that pediatric populations will likely be the ultimate vaccine target group for a prophylactic HIV-1 vaccine. We therefore evaluated the seroprevalence of and neutralizing antibody (NAb) titers to seven human Ad serotypes in pediatric subjects from sub-Saharan Africa, including Ad5, from species C; Ad11, Ad35, and Ad50, from species B; and Ad26, Ad48, and Ad49, from species D.

We first evaluated Ad5 seroprevalence in 42 paired mother-infant serum samples from Liberia to assess the magnitude of passively acquired maternal antibodies. We performed luciferase-based virus neutralization assays and calculated 90% neutralizing titers as previously described (11). As shown in Fig. 1, Ad5 seroprevalence and NAb titers in infants were remarkably high (93% seropositive, with 48% having titers of >1,000). Infant Ad5 NAb titers correlated well with maternal Ad5 NAb titers ($R^2 = 0.83$; $P < 0.0001$), and linear regression analysis demonstrated that infant titers had 78% of the magnitude of maternal titers (95% confidence interval, 67 to 90%). This correlation strongly suggests that the high Ad5 NAb titers present at birth reflect passively acquired maternal antibodies.

We next assessed the age dependence of Ad5 seroprevalence and NAb titers in pediatric populations from sub-Saharan Africa. We obtained 633 samples from subjects aged 6 months to 18 years from South Africa and divided them arbitrarily into the following age strata: 6 months to 1 year old ($n = 217$), 1 to 2 years old ($n = 39$), 2 to 7 years old ($n = 65$), 7 to 12 years old ($n = 138$), and 12 to 18 years old ($n = 174$). Five hundred one samples were obtained from South African Medical Research Council measles vaccine studies, and 132 samples were obtained from pneumonia studies at University of KwaZulu-Natal, Durban, South Africa. There were 158 individuals from whom a second sample was also obtained 6 years later. As shown in Table 1, Ad5 seroprevalence and NAb titers were markedly lower in subjects aged 6 months to 1 year (13% seropositive, with 2% having titers of >1,000) and 1 to 2 years (28% seropositive, with 3% having titers of >1,000) than in infants at birth. Ad5 seroprevalence and NAb titers then increased rapidly after age 2 and approached adult levels after age 7.

These data demonstrate a dramatic age dependence of Ad5 seroprevalence. The high Ad5-specific maternal antibody titers present at birth declined substantially by 6 months of age, which is consistent with the reported decay kinetics of measles-specific antibodies (2). We were unable to assess the precise decay trajectory of maternal antibodies, however, since we did not have samples from subjects between birth and 6 months of age. After age 2, Ad5 seroprevalence increased rapidly, presumably as a result of natural Ad5 infections. Importantly,

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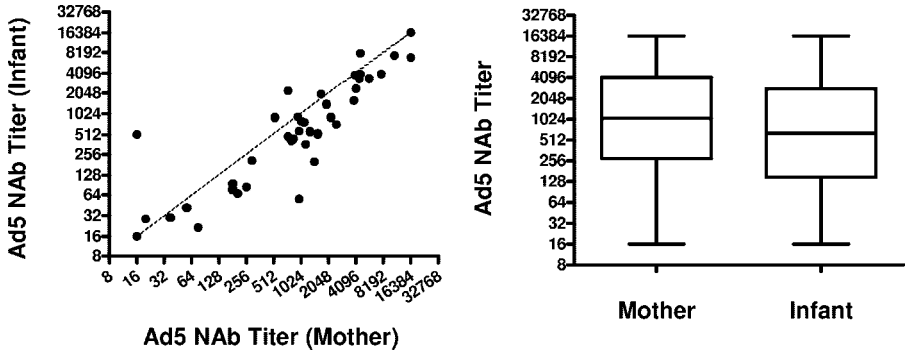


FIG. 1. Ad5 NAbs in paired mother-infant samples. Ad5 NAb titers were assessed in 42 serum samples from mothers and infants. Concordance of NAb titers between mothers and infants is shown both as a scatter plot (left) and as box-and-whisker plots (right).

there was a clear "window" of low Ad5 seroprevalence from approximately 6 months to 2 years of age, followed by a rapid rise to nearly adult levels. These data suggest that maternal Ad5-specific NAbs decay before high-titer Ad5-specific NAbs are generated by natural infections.

We next evaluated the seroprevalence of six alternative Ad serotypes from species B and D. We previously reported that the seroprevalence of Ad11 and Ad35 (species B) is substantially lower than that of Ad5 (species C) in adults (5, 6, 12, 13). We extended these observations by assessing the seroprevalence of and NAb titers to Ad11, Ad35, and Ad50, from species B, as well as Ad26, Ad48, and Ad49, from species D, in pediatric populations from South Africa. As shown in Table 1, seroprevalence and NAb titers for all of these Ad serotypes were low and showed a slow rise with age. From 6 months to 2 years of age, 6% of subjects were seropositive for Ad11, whereas <2% were seropositive for Ad35, Ad50, Ad26, Ad48, and Ad49. Among individuals over age 12, 10 to 25% were seropositive for each of these serotypes. Importantly, <2% of these subjects had NAb titers of >1,000 to Ad35, Ad50, Ad26, Ad48, and Ad49, although 10% had titers of >1,000 to Ad11.

TABLE 1. Seroprevalence and NAb titers for Ad5 (species C); Ad11, Ad35, and Ad50 (species B); and Ad26, Ad48, and Ad49 (species D)

| Vector | Patient age (yr) | % of patients with NAb titer | | | | Median NAb titer |
|---|---|---|---|---|---|---|
| | | <16 | 16–200 | 200–1,000 | >1,000 | |
| Ad5 | Birth | 7.14 | 14.29 | 30.95 | 47.62 | 512 |
| | 0.5–1 | 86.64 | 9.68 | 1.38 | 2.30 | <16 |
| | 1–2 | 71.79 | 15.38 | 10.26 | 2.56 | <16 |
| | 2–7 | 46.15 | 15.38 | 12.31 | 26.15 | 16 |
| | 7–12 | 26.81 | 14.49 | 18.12 | 40.58 | 512 |
| | 12–18 | 20.69 | 16.67 | 14.94 | 47.70 | 512 |
| Ad11 | 0.5–2 | 93.75 | 3.13 | 3.13 | 0.00 | <16 |
| | 2–7 | 85.29 | 8.82 | 2.94 | 2.94 | <16 |
| | 7–12 | 87.68 | 2.90 | 3.62 | 5.80 | <16 |
| | 12–18 | 78.74 | 8.62 | 2.30 | 10.34 | <16 |
| Ad35 | 0.5–2 | 100.00 | 0.00 | 0.00 | 0.00 | <16 |
| | 2–7 | 97.37 | 2.63 | 0.00 | 0.00 | <16 |
| | 7–12 | 90.58 | 5.07 | 3.62 | 0.72 | <16 |
| | 12–18 | 86.78 | 9.20 | 3.45 | 0.57 | <16 |
| Ad50 | 0.5–2 | 100.00 | 0.00 | 0.00 | 0.00 | <16 |
| | 2–7 | 87.18 | 12.82 | 0.00 | 0.00 | <16 |
| | 7–12 | 92.03 | 7.25 | 0.72 | 0.00 | <16 |
| | 12–18 | 88.51 | 9.77 | 1.15 | 0.57 | <16 |
| Ad26 | 0.5–2 | 98.66 | 1.34 | 0.00 | 0.00 | <16 |
| | 2–7 | 77.50 | 10.00 | 7.50 | 5.00 | <16 |
| | 7–12 | 78.26 | 12.32 | 7.25 | 2.17 | <16 |
| | 12–18 | 77.59 | 15.52 | 5.17 | 1.72 | <16 |
| Ad48 | 0.5–2 | 100.00 | 0.00 | 0.00 | 0.00 | <16 |
| | 2–7 | 90.00 | 10.00 | 0.00 | 0.00 | <16 |
| | 7–12 | 92.03 | 7.25 | 0.72 | 0.00 | <16 |
| | 12–18 | 90.23 | 8.62 | 1.15 | 0.00 | <16 |
| Ad49 | 0.5–2 | 98.66 | 1.34 | 0.00 | 0.00 | <16 |
| | 2–7 | 80.00 | 17.50 | 2.50 | 0.00 | <16 |
| | 7–12 | 81.88 | 18.12 | 0.00 | 0.00 | <16 |
| | 12–18 | 75.29 | 21.26 | 3.45 | 0.00 | <16 |

These data demonstrate that the seroprevalence and NAb titers for Ad11, Ad35, Ad50, Ad26, Ad48, and Ad49 are markedly lower than those for Ad5 in pediatric populations in sub-Saharan Africa. These serotypes are therefore potentially useful as alternative Ad vaccine vectors. Rare serotype Ad vectors, however, have proven less immunogenic than Ad5 in preclinical studies to date (1, 7, 10), and thus it will be important to evaluate the immunogenicities of these rare Ad serotypes in preclinical studies.

Our data suggest that Ad5 vectors for vaccines and gene therapy may have maximal utility when delivered during the "window" period of low Ad5 seroprevalence from 6 months to 2 years of age. An optimal and practical pediatric vaccine regimen could therefore involve priming with a replication-incompetent rare serotype Ad vector shortly after birth and boosting with an Ad5 vector during this "window" period. The safety and immunogenicity of Ad vaccine vectors in pediatric populations, however, remain to be determined.

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