Seroprevalence of Neutralizing Antibodies to Adenovirus Type 5 among Children in India: Implications for Recombinant Adenovirus-Based Vaccines

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We determined the levels of adenovirus 5 (Ad5) neutralizing antibodies in children in India less than 2 years of age. The results clearly show an age-dependent increase in Ad5-specific immunity, with 7- to 12-month-old children having the lowest levels of Ad5 immunity. This opens up the scope for the use of recombinant Ad5-based vaccines in this age group.

There are more than 50 different serotypes of adenoviruses infecting humans. These viruses can infect different cell types and thus have a wide tissue tropism range. Adenoviruses, in general, cause respiratory diseases and ocular diseases in human beings of all age groups besides causing gastrointestinal disorders in children. Most of these infections are associated with mild symptoms that are efficiently countered by the host’s immune system. A number of gene and vaccine delivery vectors have been developed on the basis of adenovirus type 5 (Ad5). Strong protective immune responses have been shown in experimental animal models against the targeted vaccine antigen expressed by using Ad5-derived recombinant viruses. Similarly, Ad5 vectors have been shown to effectively deliver target genes into the host body with therapeutic effects.

A large number of adenoviruses circulate freely in nature. As a consequence, ~80% of humans are preexposed to these viruses and are reported to have high titers of adenovirus neutralizing antibodies. This has implications for the clinical application of Ad5-based vaccines or gene therapy. However, a couple of reports have indicated that titers of Ad5 neutralizing antibodies were low in young children in Europe and sub-Saharan Africa. We have recently shown in an experimental model that low levels of Ad5 neutralizing antibodies in mice had no effect on the protective efficacy of an Ad5-derived recombinant virus expressing Japanese encephalitis virus envelope protein. This raises the possibility of using Ad5-derived recombinant vaccines for immunization of children with low Ad5 neutralizing antibody levels. Thus, understanding the level of anti-Ad5 immunity in young children in various age groups would help in the clinical application of recombinant Ad5-based vaccines against childhood infections. In the present study, we determined and compared the levels of anti-adenovirus antibodies, as well as Ad5 neutralizing antibodies, in different age groups of children in India, less than 2 years of age.

Serum samples were obtained from the blood collection center of a tertiary-care hospital during routine sampling. A total of 70 children less than 2 years of age were selected for this study, and these were divided into four groups based on age. Group 1 (n = 16) had children ≤6 months of age (mean age, 3.9 months), group 2 (n = 22) had 7- to 12-month-old children (mean age, 9.9 months), group 3 (n = 11) had 13- to 18-month-old children (mean age, 16.2 months), and group 4 (n = 21) had 19- to 24-month-old children (mean age, 23.8 months).

An enzyme-linked immunosorbent assay (ELISA) was used to determine the titers of anti-adenovirus antibodies in the serum samples. This assay, using purified Ad5 as the antigen, would capture antibodies cross-reactive to different adenovirus serotypes and thus provide a measurement of the total anti-adenovirus antibody level. Serum samples were diluted serially starting at 1:25. The reciprocal of the highest serum dilution that was positive in the ELISA was taken as the ELISA titer. The results obtained in the ELISA show an age-dependent increase in antibody titers across the groups (Table 1). In group 1, 94% of the children had anti-adenovirus antibodies whereas only 82% of the children in group 2 had adenovirus antibodies, although the geometric mean titers (GMTs) in group 1 were lower than those in group 2 (GMTs of 109 and 182, respectively). This small reduction may simply be due to the disappearance of maternally inherited antibodies over the 6-month period. Again, in groups 3 and 4 there was an increase in both the number of seropositive subjects, almost reaching 100% in both of the groups, and the levels of anti-adenovirus antibodies (GMTs of 292 and 1,229, respectively). In particular, there was a dramatic increase in anti-adenovirus immunity in children in the 19- to 24-month age group, thereby ruling out the possibility of using adenovirus-based therapeutics in children more than 18 months old. This increase is consistent with the change in the social behavior of children at this age, when they move outdoors and mix with others more frequently, thus having higher chances of adenovirus exposure.
TABLE 1. Adenovirus immunity in the children in this study

<table>
<thead>
<tr>
<th>Group</th>
<th>Age in mo (n)</th>
<th>Anti-adenovirus antibodies (ELISA)</th>
<th>Anti-Ad5 neutralizing antibodies (PRNT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GMT ± SD</td>
<td>Log₂ GMT ± SD</td>
</tr>
<tr>
<td>1</td>
<td>&lt;6 (16)</td>
<td>109.05 ± 433.88</td>
<td>4.6918 ± 1.4512</td>
</tr>
<tr>
<td>2</td>
<td>7–12 (22)</td>
<td>181.96 ± 1,351.86</td>
<td>5.2038 ± 1.5907</td>
</tr>
<tr>
<td>3</td>
<td>13–18 (11)</td>
<td>291.90 ± 663.54</td>
<td>5.6764 ± 1.6798</td>
</tr>
<tr>
<td>4</td>
<td>19–24 (21)</td>
<td>1,228.69 ± 15,041.80</td>
<td>7.1137 ± 2.5979</td>
</tr>
</tbody>
</table>

We then assayed neutralizing antibodies specific to human Ad5 in these children. A plaque reduction neutralization assay was used to determine the titer of Ad5 neutralizing antibodies in the serum samples (1). Serial dilutions of sera starting at 1:10 were used in the assay. The reciprocal of the highest dilution giving at least 50% neutralization was considered the plaque reduction neutralization titer (PRNT). Samples that gave less than 50% neutralization at a 1:10 dilution were taken as having undetectable Ad5 neutralizing antibodies. In contrast to the ELISA results, 69% of the subjects in group 1 had undetectable levels of Ad5 neutralizing antibodies. The PRNTs in the other samples ranged from 10 to 160, with a GMT of 15. Interestingly in group 2, 68% of the samples had undetectable levels of Ad5 neutralizing antibodies, and the rest of the samples had titers ranging from 10 to 40, with a GMT of 11. This observation is consistent with the ELISA results wherein, with the disappearance of the maternal antibodies, the levels of Ad5-specific antibodies decreased in the children. In group 3, the PRNTs ranged from 10 to 320, with a GMT of 16. However, both the number of seropositive cases (65%) and the Ad5-specific neutralizing antibody titers increased drastically in group 4, with PRNTs ranging from 10 to 1,280 and a GMT of 47.

A statistical analysis of the data clearly established that anti-adenovirus immunity rose in children with age. Statistical analysis was performed with the STATA 9.0 software, and differences with a P value of ≤0.05 were considered statistically significant. A one-way analysis of variance (ANOVA) of log ELISA titers among the four groups showed highly significant differences among the four groups (P = 0.0017).

We compared the ELISA titers and PRNTs of group 4 with those of the rest of the groups, as there was a substantial increase in both PRNTs and ELISA titers in children belonging to group 4. Analysis of log ELISA titers of individual groups by using Scheffe’s post-hoc ANOVA showed highly significant differences for group 1 (P = 0.005) and group 2 (P = 0.021) in comparison to group 4 (Table 2).

Thus, titers in groups 1 and 2 were significantly lower than those in group 4. Importantly, the titers in group 3 were not significantly different from those in group 4 (P = 0.274).

Similarly, highly significant differences were observed when log PRNT values were compared across the groups (P = 0.0024). Further analysis indicated significantly lower neutralizing antibody titers in group 2 (P = 0.005) in comparison to those in group 4. Further, the differences in ELISA titers (P = 0.001) and PRNTs (P = 0.0034) were highly significant when children in groups 1 and 2 were compared with those in groups 3 and 4. Thus, adenovirus immunity was significantly lower in <1-year-old children than in those in the 1- to 2-year age group.

Results presented here clearly show that children less than 12 months of age possess significantly lower anti-adenovirus antibodies and Ad5 neutralizing antibodies, after which there is a marked increase in antibody titers. In particular, after 18 months of age, anti-adenovirus titers increase drastically, indicating that children above this age may not be suitable for adenovirus-based therapies. This is consistent with earlier findings in economically developed (United States, Italy, and Germany) and underdeveloped (sub-Saharan Africa) parts of the world, where Ad5 titers were reported to be low for children less than 2 years of age, after which there was a sudden enhancement of anti-Ad5 titers (3, 4, 15, 18). Although alternate strategies are being explored to overcome the preexisting Ad5 immunity in humans (6, 21), these results, together with our earlier finding that low levels of Ad5 antibodies do not interfere with recombinant adenovirus vaccine uptake in mice (1), suggest that Ad5-based vaccines or other therapeutics may still be effective if administered to children in the 7- to 12-month age bracket.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log₂ ELISA titer</td>
<td>Log₂ PRNT</td>
<td>Log₂ ELISA titer</td>
</tr>
<tr>
<td>2</td>
<td>0.51198 (0.885)</td>
<td>−0.26387 (0.936)</td>
<td>0.47260 (0.932)</td>
</tr>
<tr>
<td>3</td>
<td>0.98458 (0.643)</td>
<td>0.05120 (1.000)</td>
<td>1.90991 (0.021)</td>
</tr>
<tr>
<td>4</td>
<td>2.42189 (0.005)</td>
<td>1.16143 (0.056)</td>
<td></td>
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</table>

*Shown are the differences in the geometric mean ELISA titers and PRNTs of different groups. The P values for the statistical significance of the differences between groups are in parentheses.*
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REFERENCES
1. Appaiahgari, M. B., M. Saini, M. Rauthan, Jyoti, and S. Vrati. 2006. Immuno- 
unization with recombinant adenovirus synthesizing the secretory form of 
Japanese encephalitis virus envelope protein protects adenovirus-exposed 
mice against lethal encephalitis. Microbes Infect. 8(2):92–104.
Rajakumar, K. N. Brown, P. D. Robbins, M. Murphy-Corb, B. R. D. Day, and 
A. Gambotto. 2006. Broad cellular immunity with robust memory responses 
to simian immunodeficiency virus following serial vaccination with adenovi-
Microbiol. 43:4426–4433.
izing antibodies against 33 human adenoviruses in normal children in Rome.
5. Gaden, F., L. Franqueville, M. Magnusson, S. S. Hong, M. D. Merten, L.
Lindholm, and P. Boulanger. 2004. Gene transduction and cell entry pathway of 
fiber-modified adenovirus type 5 vectors carrying novel endocytic 
peptide ligands selected on human tracheal glandular cells. J. Virol. 78:7227– 
7247.
D. T. Curiel. 2004. An adenovirus vector with a chimeric fiber derived from 
7. Gomez-Roman, V. R., R. H. Flores, B. Peng, D. C. Montefiori, V. S.
2006. An adenovirus-based HIV subtype B prime/boost vaccine regimen elicits 
mediating broad antibody-dependent cellular cytotoxicity against non-
cell targeted gene delivery by adenovirus 5 vectors carrying knobless fibers 
Goudsmit, and D. H. Barouch. 2006. Age dependence of adenovirus-specific 
neutralizing antibody titers in individuals from sub-Saharan Africa. J. Clin.
Microbiol. 44:3761–3783.
10. Malkevitch, N., L. J. Patterson, K. Aldrich, E. Richardson, W. G. Alvod, and 
M. Robert-Guroff. 2003. A replication competent adenovirus 5 host range mutant-simian immunodeficiency virus (SIV) recombinant priming/ 
subunit protein boosting vaccine regimen induces broad, persistent SIV-
specific cellular immunity to dominant and subdominant epitopes in Mamu-
1978. Prevalence of neutralizing antibodies against adenoviruses at Luck-
Prevalence of adenovirus types 3 and 7 antibodies in Singapore. Jpn. J. In-
13. Nwanegbo, E., E. Vardas, W. Gao, H. Whittle, H. Sun, D. Rowe, P. D.
Robbins, and A. Gambotto. 2004. Prevalence of neutralizing antibodies to 
adenoviral serotypes 5 and 35 in the adult populations of The Gambia, South 
Incidence and prevalence of neutralizing antibodies to the common adenovi-
ruses in children with cystic fibrosis: implication for gene therapy with adenovi-
15. Roth, D. A., M. D. McKirnan, I. Canestrelli, M. H. Gao, N. Dalton, N. C. Lai, 
D. M. Roth, and B. K. Hammond. 2006. Intraocular delivery of an ade-
novirus encoding fibroblast growth factor-4 in myocardial ischemia: effect of 
17:230–238.
Development of a preventive vaccine for Ebola virus infection in primates. 
17. Thorner, A. R., R. Vogels, J. Kaspers, G. J. Weaverling, L. Holtermann, A. A.
Lemckert, A. Dilraj, L. M. McNally, S. Jepsen, P. Abbink, A.
Goudsmit, and D. H. Barouch. 2006. Age dependence of adenovirus-specific 
neutralizing antibody titers in individuals from sub-Saharan Africa. J. Clin.
Microbiol. 44:3761–3783.
define neutralizing epitopes on the adenovirus hexon. J. Gen. Virol. 73:1429– 
1435.
Pavirani, M. Courtney, D. Lamy, T. Ragot, P. Saulnier, A. Andremont, R.
2002. Construction and characterization of adenovirus serotype 5 packaged 