SEROPREVALENCE OF NEUTRALISING ANTIBODIES TO ADENOVIRUS TYPE 5 IN INDIAN CHILDREN: IMPLICATIONS FOR RECOMBINANT ADENOVIRUS-BASED VACCINES

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We determined the levels of Adenovirus 5 (Ad5) neutralizing antibodies in Indian children below two years of age. The results clearly show an age-dependent increase in Ad5-specific immunity with 7-12 months 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 wide range of tissue tropism. Adenoviruses, in general, cause respiratory diseases and ocular diseases in human beings of all age groups besides causing gastro-intestinal disorders in children. Most of these infections are associated with mild symptoms that are efficiently encountered by the host’s immune system. A number of gene and vaccine delivery vectors have been developed based on Adenovirus serotypes 5 (Ad5). Strong protective immune responses have been shown in the experimental animal models against the targeted vaccine antigen expressed using the Ad5-derived recombinant viruses (1,2,7,11,17). Similarly, Ad5 vectors have been shown to effectively deliver the target genes into the host body with therapeutic effects (5,8,16).

A large number of Adenoviruses circulate freely in the nature. As a consequence, ~80% of the humans are pre-exposed to these viruses and reported to have high titers of Adenovirus neutralizing antibodies (3,9,10,12-14,18-20). This has implications for the clinical application of the Ad5-based vaccines or gene therapy. However, a couple of reports indicated that titers of Ad5 neutralizing antibodies were low in the young children from Europe (4) and sub-Saharan Africa (18). 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 (JEV) envelope protein (1). This raises the possibility of using the Ad5-derived recombinant vaccines for immunization of children with low Ad5 neutralizing antibodies. Thus, understanding the level of anti-Ad5 immunity in young children of various age groups would help in the clinical application of the recombinant Ad5-based vaccines against childhood infections. In the present study, we have determined and compared the levels of anti-Adenovirus antibodies as well as the Ad5-neutralizing antibodies in different age groups of Indian children, below 2 years of age.

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

An ELISA was used to determine the titers of anti-adenovirus antibodies in the serum samples. This assay employing the purified Ad5 as the antigen would capture antibodies cross-reactive to different adenovirus serotypes and thus provide a measure of total anti-adenovirus antibody (1). Serum samples were diluted serially starting at 1:25. Reciprocal of the highest serum dilution that was positive in the ELISA was taken as the ELISA titer. The results obtained in 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% children in group 2 had adenovirus antibodies, although, the geometric
mean titers in Group 1 were smaller compared to Group 2 (GMT of 109 & 182, respectively). This small reduction may simply be due to the disappearance of maternally-inherited antibodies over the six month period. Again, in Group 3 and 4 there was an increase in both the number of sero-positive subjects, almost reaching 100% in both the groups, as well as the levels of anti-adenovirus antibodies (GMT of 292 & 1229, respectively). Particularly, there was a dramatic increase in anti-Ad immunity in children belonging to 19-24 months age group, thereby ruling out the possibility of using Ad-based therapeutics in children above 18 months age. This increase is consistent with the change in the social behavior of children at this age when they move outdoor and mix with others more frequently, thus having higher chances of adenovirus exposure.

We then assayed neutralizing antibodies specific to human Ad5 in these children. A plaque-reduction neutralization assay was used to determine the titer of the Ad5-neutralizing antibodies in the serum samples (1). Serial dilutions of sera starting at 1:10 were used in the assay. Reciprocal of the highest dilution giving at least 50% neutralization was considered as the plaque-reduction neutralization titer (PRNT). Samples that gave less than 50% neutralization at 1:10 dilution were taken as having undetectable Ad5 neutralizing antibodies. In contrast to ELISA results, 69% of the subjects in group 1 had undetectable levels of Ad5-neutralizing antibodies. The PRNT in the other samples ranged from 10-160 with a GMT of 15. Interestingly in group 2, 68% samples had undetectable levels of Ad5-neutralizing antibodies and the rest of the samples had titers ranging from 10-40 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 PRNT titers ranged from...
10-320 with a GMT of 16. However, both the number of sero-positive cases (65%) as well as the Ad5-specific neutralizing antibody titers increased drastically in group 4 with PRNTs ranging from 10-1280 and a GMT of 47.

The statistical analysis of the data clearly established that anti-adenovirus immunity rose in children with age. Statistical analysis was performed using STATA 9.0 software and differences with \( p \)-value \( \leq 0.05 \) were considered statistically significant. One way ANOVA analysis of log ELISA titers among the four groups showed highly significant differences between the four groups (\( p=0.0017 \)). We compared the results obtained in ELISA and PRNT for group 4 with the rest of the groups, as there was a substantial increase in both PRNT and ELISA titers in children belonging to group 4. Analysis of log ELISA titers of individual groups 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, group 3 titers 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 titers in Group 2 (\( p=0.005 \)) in comparison to Group 4. Further, the differences in ELISA (\( p=0.001 \)) and PRNTs (\( p=0.0034 \)) were highly significant when children in groups 1 and 2 were compared together with those in groups 3 and 4. Thus, adenovirus immunity was significantly lower in <1 year old children than those in the 1-2 years age group.

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

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References


Table 1: Adenovirus immunity in children.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age in months (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>Log$_2$ 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 ±1351.86</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>1228.69±15041.8</td>
<td>7.1137 ± 2.5979</td>
</tr>
</tbody>
</table>

Table 2: Scheffe’s post-hoc ANOVA-based analysis of log GMTs of ELISA and PRNT across four age groups

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log$_2$ ELISA</td>
<td>Log$_2$ PRNT</td>
<td>Log$_2$ ELISA</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.51198 (0.885)</td>
<td>-0.26387 (0.936)</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>0.98458 (0.643)</td>
<td>0.05120 (1.000)</td>
<td>0.47260 (0.932)</td>
</tr>
<tr>
<td>Group 4</td>
<td>2.42189 (0.005)</td>
<td>1.16143 (0.056)</td>
<td>1.90991 (0.021)</td>
</tr>
</tbody>
</table>

Shown above are the differences in the GMTs of ELISA and PRNTs of different groups. The p-values for the statistical significance of the differences are given in the parentheses.