Effect of maternal antibody on IgA antibody response in nasopharyngeal secretion in infants and children during primary respiratory syncytial virus infection

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IgA antibody response to respiratory syncytial virus (RSV) was determined in nasopharyngeal secretions (NPS) of 22 infants and children infected with RSV group A strains, employing an ELISA. The antibody activity observed during the convalescent phase against whole virus, fusion glycoprotein (F) and large glycoprotein (G) was examined in young infants (under 6 months) and compared with that of older individuals (6 to 16 months). Both groups showed similar degrees of IgA antibody activity to whole virus in NPS; however, older individuals showed a significantly higher activity of IgA F antibody than that of IgA G antibody in the NPS. On the other hand, in the NPS of young infants, IgA F antibody was somewhat suppressed and IgA G antibody activity predominated over that of IgA F. Pre-existing (maternal) serum IgG anti-RSV F antibody activity was higher than that of antibody to G. A significant reverse correlation was observed between the activity of pre-existing serum IgG F antibody and NPS IgA F antibody in the convalescent phase after primary infection with RSV. These observations suggest that maternally derived RSV IgG antibody, which contains abundant anti-F activity, may suppress the development of IgA F antibody response at infection sites in the respiratory tract in young infants during primary RSV infection. These changes may be related to the severity of acute infection and longer convalescence often observed in young infants during RSV infection.

Respiratory syncytial virus (RSV) is unique among respiratory pathogens because of its predilection for causing serious lower respiratory tract disease among infants and children under 4 years. In the youngest age groups, pneumonia and bronchiolitis develop in the presence of maternally derived antibody (Kim et al., 1973; McIntosh & Chanock, 1987). Because RSV manifests extremely restricted tropism for the respiratory epithelium and spreads only rarely to extramucosal sites, development of virus-specific local immunity, particularly a local secretory IgA response, has been thought to have a significant protective role for infection (McIntosh et al., 1978; Kaul et al., 1981; Welliver et al., 1989; Nadal & Ogra, 1990).

Recent studies on the immune response in infants and children undergoing primary RSV infection have demonstrated that infants less than 9 months produce less antibody to both the fusion glycoprotein (F) and large glycoprotein (G) than do older infants and children (Murphy et al., 1986a,b). Both immunological immaturity and immunosuppression mediated by maternally derived antibodies are presumed to be responsible for the poor immune response of the young infants (Parrott et al., 1973; Murphy et al., 1986a,b). However, the mechanism of such immunomodulation in the respiratory tract during RSV infection remains to be defined.

In this study, infants and children during primary infection with RSV group A strain were examined for the development of IgA F or IgA G antibody responses in nasopharyngeal secretions (NPS). The effect of maternal IgG antibody on local IgA antibody production during the acute phase of infection with RSV was explored.

Twenty-two infants and children, 19 days to 16 months of age, infected with RSV group A strain were enrolled in this study. These subjects did not have underlying immunological diseases. The identification and group determination of viruses isolated were done with tissue culture ELISA using several group A- and B-specific monoclonal antibodies (MAbs) (Tsutsumi et al., 1989; Nagai et al., 1993). Of the 22 individuals, all were febrile during acute illness, seven had bronchitis, 11 had bronchiolitis, and four had pneumonia. Serum and NPS
samples were collected during the acute phase (3 to 6 days) and convalescent phase (12 to 18 days after onset) of infection, and frozen until further examination.

The amount of total IgA present in NPS specimens was determined by ELISA. A 100 μl sample of alpha-chain-specific goat anti-human IgA (Tago) diluted in carbonate buffer (pH 9.6) was added to the wells of round-bottomed, polyvinyl microELISA plates (Dynatech Laboratories). After overnight incubation at 4 °C, the plates were incubated at room temperature with 1% BSA for 2 h and were then washed once with 0.02% Tween-20 in PBS (PBS-Tween). Purified human IgA (ICN ImmunoBiologicals) was used as the standard. The human IgA and NPS specimens were diluted in 1% BSA–PBS and 0.1 ml was added to each well. The plates were incubated at 37 °C for 2 h and washed four times before 0.1 ml of anti-human IgA peroxidase conjugate (from MBL) was added to each well. The plates were incubated for 2 h at 37 °C and washed three times, and then 0.1 ml of substrate (o-phenylenediamine dihydrochloride) was added to each well. After 15 min, 125 m- H2SO4 was added and A490 was measured for each well. The concentration of IgA in NPS was determined from the graph of A490 versus concentration generated with the human IgA standard.

The determination of antibody response in serum and NPS to whole virus, F and G proteins was performed by tissue culture ELISA. The antibody to whole RSV was assayed using a HEp-2 cell monolayer infected with RSV strain Long (prototype group A strain) as the solid-phase antigen, and uninfected HEp-2 cells were used as control. HEp-2 cells were infected with RSV strain Long at a multiplicity of 0.2. When the infected HEp-2 cells showed about 80% c.p.e., the cells in the microplates were washed with PBS-Tween and fixed with 80% (v/v) acetone–PBS for 15 min at 4 °C. For determination of antibody activity to RSV F or G, HEp-2 cells infected with vaccinia virus-expressed F of RSV strain A2 (prototype group A strain) (vaccinia-A2-F) or vaccinia virus-expressed G of RSV A2 strain (vaccinia-A2-G) were used. Vaccinia virus-infected HEp-2 cells were used as controls. The recombinant viruses and the vaccinia virus were inoculated at a multiplicity of 0.5. These viruses were kindly provided by Dr R. A. Olmsied (NIH) (Olmsted et al., 1986).

The recombinant virus and the vaccinia virus-infected monolayers were fixed with acetone by the same method used for the Long strain-infected HEp-2 cells when the cells showed almost 100% c.p.e. The specificity of expression of F or G in the recombinant virus-infected cells was confirmed by ELISA with MAbs specific for F, G, nucleoprotein (NP), phosphoprotein (P) and the 22K protein (Tsutsumi et al., 1989; Nagai et al., 1993).

Acetone-fixed microplates were blocked with 1% BSA–PBS for 2 to 3 h at room temperature and washed once with PBS–TWEEN. Then, 0.1 ml samples of serum and NPS serially diluted twofold with 0.2% BSA–PBS were added in duplicate to the wells and incubated for 1 h at 37 °C. After washing four times, peroxidase-labelled goat anti-human IgA or IgG (MBL) was added. The samples were incubated for 1 h at 37 °C and substrate (o-phenylenediamine dihydrochloride) was added to each well. Fifteen minutes later, the reaction was stopped with 1.25 m-H2SO4 and the A490 was measured. Specific absorbance was calculated by subtracting the mean absorbance of two wells with uninfected or vaccinia virus-infected cells from the mean absorbance of two wells with Long strain or recombinant virus-infected cells. The ELISA titres were expressed as the highest dilution that gave specific absorbance over 0.1. The ELISA IgA and IgG titres in NPS were corrected to 0.1 mg/ml total IgA content.

To ascertain the specific expression of F and G in recombinant virus-infected HEp-2 cells, MAbs (mouse ascites) specific for RSV F, G, NP, P and the 22K protein were tested in ELISAs against the HEp-2 cells infected with Long strain, 58-17 strain (group B), vaccinia-A2-F or vaccinia-A2-G. The procedures for ELISA were the same as those described for assay of serum and NPS antibody activity, except that the anti-mouse IgG peroxidase conjugate was used as the second antibody. Long strain-infected monolayers reacted to group A-specific and group-common MAbs, and 58-17 strain-infected monolayers to group B-specific and group-common MAbs. The amount of total IgA present in NPS specimens was determined by ELISA. A 100 μl sample of alpha-chain-specific goat anti-human IgA (Tago) diluted in carbonate buffer (pH 9.6) was added to the wells of round-bottomed, polyvinyl microELISA plates (Dynatech Laboratories). After overnight incubation at 4 °C, the plates were incubated at room temperature with 1% BSA for 2 h and were then washed once with 0.02% Tween-20 in PBS (PBS-Tween). Purified human IgA (ICN ImmunoBiologicals) was used as the standard. The human IgA and NPS specimens were diluted in 1% BSA–PBS and 0.1 ml was added to each well. The plates were incubated at 37 °C for 2 h and washed four times before 0.1 ml of anti-human IgA peroxidase conjugate (from MBL) was added to each well. The plates were incubated for 2 h at 37 °C and washed three times, and then 0.1 ml of substrate (o-phenylenediamine dihydrochloride) was added to each well. After 15 min, 125 m- H2SO4 was added and A490 was measured for each well. The concentration of IgA in NPS was determined from the graph of A490 versus concentration generated with the human IgA standard.

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The NPS IgA and IgG antibody responses to whole virus in primary infection with RSV were determined
Table 2. NPS IgA and IgG antibody response of infants and children undergoing primary infection with RSV as determined by tissue culture ELISA

<table>
<thead>
<tr>
<th>Age (months) (no. tested)</th>
<th>IgA antibody</th>
<th>IgG antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute</td>
<td>Convalescent</td>
</tr>
<tr>
<td>0 to &lt; 6 (n = 12)</td>
<td>1.4 ± 0.2</td>
<td>8.4 ± 0.6</td>
</tr>
<tr>
<td>6 to &lt; 16 (n = 10)</td>
<td>18 ± 0.5</td>
<td>9.7 ± 0.5</td>
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</tbody>
</table>

* The NPS ELISA titres were corrected to 0.1 mg/ml total IgA. The lowest dilution tested in the ELISA was 1:4. NPS specimens without detectable ELISA antibody were assigned a titre of 1:2 for calculation of mean titres.
†† Student’s t-test, P < 0.05.

Table 3. NPS IgA and serum IgG antibody response of infants and children during acute and/or convalescent phase of primary RSV infection determined by tissue culture ELISA

<table>
<thead>
<tr>
<th>Age (months) (no. tested)</th>
<th>NPS (IgA)*</th>
<th>Serum (IgG)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute</td>
<td>Convalescent</td>
</tr>
<tr>
<td>0 to &lt; 6 (n = 12)</td>
<td>10 ± 0.0</td>
<td>56 ± 0.8‡</td>
</tr>
<tr>
<td>6 to &lt; 16 (n = 10)</td>
<td>10 ± 0.0</td>
<td>84 ± 0.6</td>
</tr>
</tbody>
</table>

* The NPS ELISA titres were corrected to 0.1 mg/ml total IgA. The lowest dilution of NPS tested in the ELISA was 1:100. NPS specimens without detectable ELISA antibody were assigned a titre of 1:2 for calculation of mean titres.
† The lowest dilution of serum tested in the ELISA was 1:100. Serum specimens without detectable ELISA antibody were assigned a titre of 1:50 for calculation of mean titres.
‡ Student’s t-test, P < 0.05.
§ Student’s t-test, P < 0.01.
¶ Student’s t-test, P < 0.005.

(Table 2). All patients had a significant (over fourfold) rise in IgA antibody response during the convalescent phase of infection. Young infants (19 days to 6 months) exhibited a similar magnitude of NPS IgA response to that of the older individuals (6 to 16 months). Young infants had significant IgG antibody levels in NPS in the acute phase (presumably of maternal origin), whereas older individuals exhibited little or no IgG in NPS in the acute phase (P < 0.05). On the other hand, in the convalescent phase, only six of 12 young infants developed a significant IgG response, whereas all 10 of the older individuals did, and the difference of the mean titre between the two age groups was significant (P < 0.05).

The IgA F and G antibody responses in NPS in each age group were determined as shown in Table 3. In the acute phase, neither age group exhibited an IgA F or G antibody response in the NPS. However, in the convalescent phase, 10 and 11 (of 12) young infants developed IgA F and IgA G antibodies respectively. The mean titre of the IgA F antibody was significantly lower than that of IgA G antibody (P < 0.05). On the other hand, all and seven of 10 older individuals developed IgA F and IgA G antibodies respectively and the mean titre of the IgA F antibody response was predominant (P < 0.005).

The serum IgG F and IgG G antibody activities in the acute phase in all the 22 infants and children were examined to analyse the immunomodulatory effect of maternally derived IgG antibody on development of the NPS IgA response (Table 3). In older individuals, there were low levels of IgG F or IgG G antibodies in serum; however, young infants displayed significant serum IgG F and G antibody activities. The mean IgG F antibody
activity was significantly higher than that of IgG G antibody ($P < 0.01$).

There was a statistically significant reverse correlation between the responses of the serum IgG F antibody during the acute phase and that of NPS IgA F antibody in the convalescent phase of primary infection with RSV ($r = -0.53, 0.01 < P < 0.02$) when the specimens from all the 22 infants and children were analysed (Fig. 1). However, there was no correlation between the levels of serum IgG G antibody in the acute phase and the titre of NPS IgA G antibody in the convalescent phase ($r = -0.04, P > 0.5$) (data not shown).

In this study, we have demonstrated the induction of specific RSV IgA (whole virus), IgA F and IgA G antibody activity in NPS during primary RSV infection using a simple and sensitive tissue culture ELISA.

This is the first observation of an age-dependent alteration in the proportions of IgA antibodies against virus structural proteins. The IgG antibody against F was dominant in pre-existing (maternal) serum and a statistically significant reverse correlation was observed between the amount of pre-existing IgG F antibody and the subsequent development of NPS IgA anti-F activity during convalescence. These observations suggest that maternal IgG F antibody may suppress the development of the IgA F antibody activity in the NPS of infants. In this study, almost all cases under 6 months of age had an RSV infection sufficiently severe to require hospitalization, suggesting that the maternal antibody itself may not protect against illness. However, such maternal IgG antibody might decrease the presentation of RSV F antigen to the infant's mucosal immune system by neutralizing the F antigen at the mucosal site.

Earlier studies by Parrott et al. (1973) showed that the level of pre-existing (maternal) serum neutralizing antibody was inversely related to the height of the serum CF antibody response during convalescence. Murphy et al. (1986a, b) analysed serum IgG and IgA antibody against RSV F and G of infants and children during primary RSV infection with ELISA using affinity column-purified F and G proteins. They demonstrated a positive correlation between age and serum IgA F antibody, and a reverse correlation between pre-existing serum IgG antibody to G and serum IgA G response. There were several differences between Murphy's study and ours. They analysed serum IgA antibodies, not NPS IgA antibodies, as in this study; in addition, these studies did not distinguish between patients infected with group A and those with group B. The degree of antigenic relatedness between F proteins of groups A and B has been estimated to be 50%, and between G proteins of groups A and B, only 5% (Johnson et al., 1987; Johnson & Collins, 1988). Hence it may be necessary to distinguish between the virus groups to analyse antibody activity against virus structural proteins.

RSV F protein is now considered to be a major and important protein involved in viral infectivity (Walsh et al., 1985, 1986). Suppression of the development of IgA F antibody response in respiratory mucosa of young infants may partially explain the high vulnerability to RSV infection in this age group. The use of immunoglobulin administered parenterally during the acute phase of RSV infection (Hemming et al., 1987) may similarly suppress the development of NPS IgA F and may relate to the high susceptibility to re-infection. Immunization of young infants with F subunit vaccine alone (Belshe et al., 1993), which induces serum IgG F antibody, may bring about a similar suppressive effect on the local immune system during primary natural infection with RSV. The implications of these findings may be relevant to RSV vaccine development strategy.

References


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