Immunoglobulin G Antibody Avidity in Patients with Respiratory Syncytial Virus Infection

O. MEURMAN, M. WARIS, AND K. HEDMAN

Department of Virology, University of Turku, 20520 Turku, and Department of Virology, University of Helsinki, 00290 Helsinki, Finland

Received 8 January 1992/Accepted 13 March 1992

The titer and avidity of respiratory syncytial virus-specific antibodies were measured in 196 serum specimens from 93 children with an acute, laboratory-confirmed respiratory syncytial virus infection. An enzyme immunoassay method based on the ability of urea to dissociate the bound antibodies with low avidity from the antigen was used. Three patterns of immune responses were observed. Children <6 months of age usually had low titers of antibodies with high avidity in their acute-phase serum samples. These antibodies were concluded to be of maternal origin, since their reaction pattern was similar to that of healthy adults. During the next few weeks, a slight increase in titers with a concurrent decrease in antibody avidity was observed. All children 6 to 24 months of age had low-avidity antibodies in their acute-phase serum samples, which matured to high avidity during the follow-up. On the contrary, about half of the children >24 months of age had high-avidity antibodies already in the acute-phase serum samples. We conclude that the former children were experiencing primary infections with respiratory syncytial virus and the latter were experiencing reinfections. All adults with remote immunity had antibodies with high avidity.

During an acute infection, the maturation of immune response is characterized by both quantitative and qualitative changes in specific antibodies. The latter include, e.g., changes in the immunoglobulin class distribution and the affinity of the antibodies (i.e., the strength of the antibody-antigen bond). Affinity or, in case of multivalent antigens, antibody avidity previously could only be measured by laborious competition techniques (see reference 29) or by mathematical analysis of enzyme immunoassay titration curves (17). Recently, simple immunoassays to measure antibody avidity have been published. Protein-denaturing agents (e.g., diethylamine, isothiocyanate, and urea) are either included in the serum diluents to prevent the binding of low-avidity antibodies (13, 28) or used in the washing buffer to elute the bound low-avidity antibodies from the antigen (3, 10, 15, 27). With these methods, avidity measurement has found important applications in the diagnosis of several infections, including rubella and toxoplasmosis and varicella-zoster virus infection (9, 10, 15, 28).

Respiratory syncytial virus (RSV) is the most important pathogen causing severe lower respiratory tract infections in infants and small children (1). Its special features include the incomplete protection of infants by maternal antibodies, frequent reinfections, and the possible role of antibodies in the pathogenesis of the disease. In this study, we have analyzed the immune response after acute RSV infection with respect to antibody titer and avidity.

MATERIALS AND METHODS

Serum specimens. Altogether, 196 serum samples from 93 patients with laboratory-confirmed RSV infections were tested. The diagnosis was based on the detection of RSV antigens in nasopharyngeal secretions. The mean age of the patients was 12.5 months (range, 1 to 58 months). Of the patients, 24 were 0 to 3 months of age, 15 were 4 to 6 months, 24 were 7 to 12 months, 14 were 13 to 24 months, and 16 were >24 months of age. In 43 patients, the subgroup of the infecting RSV strain was determined. A subgroup A virus was detected in 35 patients, and a subgroup B virus was detected in 8 patients. Acute- and convalescent-phase serum specimens were collected from all infants and children.

As a control group, serum samples from 85 laboratory workers (mean age, 38 years; range, 22 to 60 years) were included. Paired specimens collected ≥2 years apart were selected. The specimens were screened for RSV antibodies, and if found positive, the later serum sample of each pair was used as a control representing remote immunity.

Viral antigen preparation. RSV subgroup A (Randall strain) and RSV subgroup B (WV3212 strain) were grown in Vero cells with Eagle’s minimal essential medium supplemented with 2% fetal calf serum and antibiotics. When an extensive cytopathic effect was observed, the cells were scraped off into maintenance medium and pelleted by centrifugation (8,000 rpm for 20 min at 4°C). The pellets were resuspended in a small volume of phosphate-buffered saline, pH 7.3 (PBS), and homogenized by a Dounce homogenizer (30 strokes). Cell debris was removed by low-speed centrifugation, and the supernatant was ultracentrifuged at 30,000 rpm for 1 h with a Beckman 50.2 Ti rotor. The pellets were resuspended in a small volume of PBS, disrupted by sonication, and used as the antigen in the enzyme immunoassay. The optimal amount of subgroup A antigen as determined by cross titration was 0.1 µg of protein per well. Group A antigen was used in all experiments if not stated otherwise.

Enzyme immunoassays. The enzyme immunoassay for the avidity of anti-RSV immunoglobulin G (IgG) was a modification of the method of Hedman et al. (8). The antigens, as dissolved in 8 M urea in PBS (PBS-urea), were adsorbed onto the wells of polystyrene microtiter strips (Microstrip; Eflab, Helsinki, Finland). After overnight incubation at room temperature, the strips were washed twice with PBS-urea and then with PBS containing 0.05% Tween 20 (PBS-Tween) and used immediately.

Serum specimens were diluted serially at fourfold steps from 1:100 to 1:25,600 in PBS-Tween, and 100 µl was added.
to the wells of microtiter strips. After 2 h of incubation at 37°C, the wells were washed three times (5 min each) either with PBS-Tween or with PBS-Tween containing 8 M urea (PBS-Tween-urea). Thereafter, 100 µl of a 1:1,000 dilution of horseradish peroxidase-labeled swine antibodies against human IgG (Orion Diagnostica, Espoo, Finland) in PBS-Tween was added to each well. The wells were incubated at 37°C for 1 h and washed three times with PBS-Tween, and 100 µl of fresh substrate solution (0.3% 1,2-phenylenediamine and 0.02% hydrogen peroxide in citrate-phosphate buffer [pH 5.5]) was added. The reaction was stopped after 30 min of incubation by adding 150 µl of 1 M hydrochloric acid to each well. The $A_{492}$ was read with an automatic eight-channel spectrophotometer (Titertek Multiscan; Labsystems, Helsinki, Finland).

The endpoint titer was defined as the dilution at which the absorbance of the serum was three times that of a negative control or at least 0.15 absorbance units. The avidity of the antibodies was expressed as the ratio of endpoint titers obtained after washing with PBS-Tween-urea and after washing with PBS-Tween alone (Fig. 1).

**Antigen detection.** RSV antigen in nasopharyngeal secretions was detected by a time-resolved fluoroimmunooassay as described by Waris et al. (33). The time-resolved fluoroimmunoassay was also used for subgroup determination of the RSV antigens (32).

**RESULTS**

**Antibody titer and avidity in remote RSV infections.** All 85 laboratory workers had RSV antibodies in both serum specimens as an indication of an RSV infection contracted earlier. The later serum samples, taken 1 to 12 years (mean, 4 years) after the first ones, were chosen as controls representing remote immunity. The mean RSV titer of these serum samples was 6,630 (range, 800 to 30,400), and the mean avidity was 80% ± 14% (range, 54 to 100%).

**Antibody titer and avidity in acute RSV infections.** All 24 patients aged 0 to 3 months had low levels (mean titer, 640) of RSV antibodies in the acute-phase serum samples taken 0 to 10 days after the onset of infection. The avidity of these antibodies was high (mean, 77%), with a level comparable to those of the antibodies from adults with remote infections (Table 1). During the next few weeks, a small rise in antibody titer was observed, accompanied by a marked decrease in avidity for most infants (Table 1).

At 4 to 6 months of age, 9 of 15 patients had low levels of high-avidity antibodies in their acute-phase serum samples, which was followed by an increase in the titer and a decrease in the avidity in subsequent specimens (Table 1). A late-convalescent-phase serum sample was available for only one of these nine infants. In this case, an increase in avidity following the previous decrease was observed. The remaining six patients of the group aged 4 to 6 months as well as all 38 patients aged 7 to 24 months had in their acute-phase serum samples either no antibodies (15 patients) or low levels of low-avidity antibodies. In all these patients, a rise in both antibody titer and avidity occurred in subsequent specimens (Table 1).

Of the patients who were >24 months of age, 8 of 16 also showed this type of antibody response, whereas the other half had a relatively high level of high-avidity antibodies already in the acute-phase serum samples. Later in infection, a further increase in both antibody titer and avidity was observed. The titers and avidity levels observed at 20 to 50 days after onset of infection were markedly higher than those in the other groups of patients and comparable to those of the remote-immunity group (Table 1).

Figures 2 and 3 show the IgG titers and avidities of all specimens plotted against time.

**Antibody response to RSV groups.** The antigen concentrations were first standardized to give equal titers with serum samples from laboratory workers representing remote immunity. Comparable results were obtained with 0.1 µg of subgroup A antigen and 0.15 µg of subgroup B antigen per well. Serum samples from seven children with a subgroup A infection and from six children with subgroup B infections were then tested against both subgroup A and B antigens (Table 2).

Serum samples from children with subgroup A infections and also those from children with subgroup B infections showed stronger reactivity against subgroup A than against subgroup B antigens. In the convalescent-phase serum samples, the titers with subgroup A antigen were on an average 2.1 and 2.0 times higher than those with subgroup B antigen for patients infected with subgroup A and B viruses, respectively. A clear difference was seen in the avidity results. In the patients with subgroup A infections, the avidity of the convalescent-phase antibodies was 4.1 times higher for subgroup A antigen than for subgroup B antigen. In the patients with subgroup B infections, the avidity for subgroup A antigen was only 1.4 times higher than that for subgroup B antigen.

**DISCUSSION**

In the present study, the maturation of antibody avidity in RSV infection followed a pattern similar to that observed in...
rubella infection (3, 10, 27, 28). In primary infections, IgG avidity was low during the first 1 to 2 months and, in a small number of patients who were followed up for a longer period, reached values comparable to those observed in the remote-immunity group in 2 to 5 months. The decrease in antibody avidity observed in small infants during the first 2 months was concluded to be due to the replacement of maternal high-avidity antibodies with the infant's own low-avidity antibodies. Unfortunately, further follow-up serum samples from these infants were not available to verify the subsequent maturation of antibody avidity.

The role of antibodies in RSV infection is controversial. On one hand, the antibodies have been speculated to be involved in the pathogenetic mechanism of the disease (18), as severe disease is most common during the first 6 months of life, when maternal antibodies are present. Human sera containing RSV antibodies have also been shown to enhance RSV infections in macrophage cell lines in vitro (5, 16). On the other hand, there is both clinical (4, 6) and experimental (26) evidence indicating that maternal antibodies offer at least partial protection against the disease.

The immune response to RSV is age dependent; in young infants, both the cell mediated (2) and humoral (19, 23, 25, 31) responses are weak. This decreased response involves both fusion protein F and the surface glycoprotein G (19, 23, 25, 31). Also in this study, the convalescent-phase antibody titers of the infants remained markedly lower than those of the children aged >6 months. On the contrary, the rate of the increase in antibody avidity was not age dependent. Maternal antibodies, which have been reported to suppress anti-
body production both in infants and in experimental animals (23, 24), could not be shown to suppress the maturation of IgG avidity. However, this requires further verification, since because of the short follow-up, a subsequent increase in avidity after the previous decrease could be observed in only one infant.

In humans, circulating IgG antibodies do not protect from reinfections with RSV, which are common in spite of even high antibody titers in serum (7, 11). From our results, it is evident that this incomplete protection is not caused by low antibody avidity.

Based on reactivity with monoclonal antibodies, RSV can be divided into two groups, designated A and B (22). When purified glycoproteins are used as antigens, antibody responses to the fusion protein show extensive cross-reactivity, whereas responses to the G protein of the heterologous strain are markedly weaker than those to the homologous strain (12, 31). The whole-virus antigens used in this study allowed no proper comparison of avidity with strain variation, although there was evidence suggesting that antibodies bind to the homologous strain with higher avidity than to the heterologous strain. In general, reactivity against subgroup A antigen was stronger than that against subgroup B antigen. Interestingly, Muulenaer et al. (21) showed recently that primary infections with subgroup A virus elicited cross-reactive antibodies to both the G and F glycoproteins of

FIG. 3. Anti-RSV IgG antibody avidity in 193 serum samples from 92 patients with an acute RSV infection.
TABLE 2. RSV antibody titer and avidity tested against antigens prepared from subgroup A and B viruses

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time of sampling (days after infection)</th>
<th>Infecting group</th>
<th>RSV subgroup A*</th>
<th>RSV subgroup B*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Titer</td>
<td>Avidity (%)</td>
</tr>
<tr>
<td>No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>9</td>
<td>A</td>
<td>&lt;100</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td></td>
<td>1,100</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>4</td>
<td>A</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td></td>
<td>6,400</td>
<td>50</td>
</tr>
<tr>
<td>1</td>
<td>19</td>
<td>2</td>
<td>A</td>
<td>&lt;100</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td></td>
<td>1,800</td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td>7</td>
<td>A</td>
<td>600</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td></td>
<td>3,800</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>7</td>
<td>A</td>
<td>1,700</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td></td>
<td>4,900</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>4</td>
<td>A</td>
<td>&lt;100</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td></td>
<td>3,200</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>5</td>
<td>A</td>
<td>2,400</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td></td>
<td>13,700</td>
<td>81</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>8</td>
<td>B</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td></td>
<td>1,600</td>
<td>14</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>6</td>
<td>B</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td></td>
<td>1,100</td>
<td>23</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>6</td>
<td>B</td>
<td>&lt;100</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>900</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>19</td>
<td>8</td>
<td>B</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td></td>
<td>4,900</td>
<td>25</td>
</tr>
<tr>
<td>13</td>
<td>25</td>
<td>3</td>
<td>B</td>
<td>&lt;100</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td></td>
<td>1,200</td>
<td>22</td>
</tr>
<tr>
<td>12</td>
<td>58</td>
<td>2</td>
<td>B</td>
<td>1,100</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td></td>
<td>5,200</td>
<td>100</td>
</tr>
</tbody>
</table>

* — not determined; values for the homologous subgroup are in boldface.

In rubella, varicella-zoster virus infection, and toxoplasmosis, the detection of low-avidity antibodies can be used for rapid diagnosis of acute infection from a single serum specimen and for distinguishing primary infections from reinfections (9, 10, 14, 15, 28). With RSV, however, the diagnostic applications appear less cut-off. In young infants, the weakness of antibody responses makes antigen detection methods more useful than serology (20, 33), and in older children with many reinfections, avidity measurement does not bring universal advantages over conventional serological methods. Although a positive finding (detection of either low-avidity antibodies or a significant change in antibody avidity) can be considered diagnostically significant, a negative finding does not exclude a recent infection. Also with RSV, avidity measurement can be used for distinguishing primary infections from reinfections.

Another possible application of avidity measurements is in future RSV vaccine studies, in which it might be of interest to measure the qualitative differences in antibody responses elicited by different vaccine preparations.

REFERENCES


