Serum Immunoglobulin G Antibody Subclass Responses to Respiratory Syncytial Virus F and G Glycoproteins after Primary Infection

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Because the immunoglobulin G (IgG) response to carbohydrate antigens is typically from the IgG2 subclass and the IgG response to protein antigens is typically from the IgG1 and sometimes the IgG3 subclass, two respiratory syncytial virus glycoproteins, F and G, which differ substantially in the amount of glycosylation, were used as antigens in an enzyme-linked immunosorbent assay to determine IgG subclass responses in 20 infants and young children with naturally acquired respiratory syncytial virus infection. Both glycoproteins elicited primarily IgG1 and IgG3 responses, indicating that the protein moieties of the glycoproteins may be immunodominant in this age group.

Respiratory syncytial virus (RSV), which is a negative-stranded RNA virus belonging to the pneumovirus subgroup of the paramyxovirus family, has two virion membrane glycoproteins that are the major protective antigens of the virus (4, 7, 18, 19, 21; M. Satake, J. E. Coligan, N. Elango, E. Norby, and S. Venkatesan, Nucleic Acids Res., in press). One of these, referred to as the F (or fusion) glycoprotein, has an estimated molecular size of 70,000 daltons and has the structure of a typical paramyxovirus glycoprotein (2, 3). The second protein is the G glycoprotein, which has an apparent molecular size of 90,000 daltons as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (21; Satake et al., in press). Interestingly, the unglycosylated G protein has a molecular size of only 32,000 daltons, suggesting that the carbohydrate content of the G glycoprotein might be substantial, although the number of side chains and molecular size of the carbohydrate have not been determined. It was recently reported that children undergoing primary infection with RSV differ in their immune response to the F and G glycoprotein, the latter being a poor immunogen (20). Ward et al. suggested that the poor response to the heavily glycosylated G protein may be a result of its being seen by the immune system as a polysaccharide antigen; i.e., the response is primarily an immunoglobulin G2 (IgG2) and IgG4 response that matures slowly during a child's infancy (12). It was recently shown that infants and children can elaborate IgG antibody to the G protein after primary RSV infection (11). This fact permitted us to further characterize the IgG subclass distribution of this response to determine whether it is more typical of a viral (predominantly IgG1 and IgG3) or polysaccharide (predominantly IgG2 and IgG4) antigen.

The sera used in this study were obtained from eight children infected with RSV who were enrolled in the Vanderbilt Vaccine Clinic, Nashville, Tenn., and 12 infants and children hospitalized at Children’s Hospital, Washington, D.C., with either primary RSV bronchiolitis or pneumonia (11, 13). In each case infection was documented by the isolation of RSV from the patient. Serum samples from the former group were collected before and after infection as previously described (11), and serum samples from the hospitalized infants and children were collected at the time of admission (when the infection was acute) and approximately 3 weeks later (when the patient was convalescent) (13). The 20 patients were selected from a larger group of patients based on the following. (i) They were old enough not to have high levels of maternal IgG antibody, although some antibody was present in the younger infants. (ii) They were determined to have rises in total IgG antibody titer to RSV F and G glycoproteins as determined by an enzyme-linked immunosorbent assay (11). In this group of 20 patients, 8 were males and 12 were females, and ages ranged from 4 to 21 months (mean, 13 months). Eleven patients had bronchiolitis, four patients had pneumonia, three patients had otitis media, one patient had croup, and one patient had rhinitis.

The RSV F and G glycoproteins were purified by immunoaffinity chromatography as previously described (18, 19). The murine monoclonal antibodies to human IgG subclasses (HP6001, αlgG1; HP6014, αlgG2; HP6048, αlgG3; HP6023, αlgG4) were produced and tested for specificity as previously described (14). In addition testing, purified myeloma proteins of IgG1, IgG2, IgG3, and IgG4 subtypes were used to coat 96-well round-bottomed plates (Immulon-1; Dynatech Laboratories, Inc., Alexandria, Va.) with 100 ng of protein per ml. Each murine anti-subclass antibody was tested at various dilutions against each of the myeloma proteins. At the dilution of antibody used in this study, no significant cross-reactivity with myeloma proteins outside the specific IgG subclass was recognized by the monoclonal antibody (data not shown).

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TABLE 1. IgG subclass response to RSV F and G glycoproteins for 20 infected infants and children

<table>
<thead>
<tr>
<th>Glycoprotein</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of infants studied</td>
<td>F</td>
<td>G</td>
<td>F</td>
<td>G</td>
</tr>
<tr>
<td>% Patients with fourfold rise in antibody titer</td>
<td>Reciprocal mean log 2 titer ± SEM</td>
<td>% Patients with fourfold rise in antibody titer</td>
<td>Reciprocal mean log 2 titer ± SEM</td>
<td>% Patients with fourfold rise in antibody titer</td>
</tr>
<tr>
<td>Preinfection</td>
<td>Postinfection</td>
<td>Preinfection</td>
<td>Postinfection</td>
<td>Preinfection</td>
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<td>----</td>
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</tr>
<tr>
<td>F</td>
<td>20</td>
<td>100</td>
<td>5.1 ± 0.4</td>
<td>10.5 ± 0.5</td>
</tr>
<tr>
<td>G</td>
<td>20</td>
<td>85</td>
<td>4.1 ± 0.4</td>
<td>7.0 ± 0.5</td>
</tr>
</tbody>
</table>

The enzyme-linked immunosorbent assay for detection of subclass antibody responses to the F and G glycoprotein of RSV was performed as described previously for total IgG (11), with the following modifications. After coating plates with antigen and serially diluting the serum specimens fourfold, we added a 1:20,000 dilution of anti-subclass antibody. The plates were incubated for 4 h and washed, and 75 µl of a 1:100,000 dilution of fluorescein isothiocyanate-conjugated goat anti-mouse antibody (Coulter Corp., Hialeah, Fla.) was added to all wells. The plates were then incubated overnight and washed. 75 µl of a 1:750 dilution of alkaline phosphatase-conjugated rabbit anti-fluorescein isothiocyanate (22) was added. The plates were incubated an additional 4 h and washed. 75 µl of p-nitrophenyl phosphate (1 mg/ml; Sigma Chemical Co., St. Louis, Mo.) was added, and the A405 of the wells at 1 h was determined with an enzyme-linked immunosorbent assay reader (Titertek; Flow Laboratories, Inc., McLean, Va.). Wells with an optical density of twofold or more above background absorbance in uncoated wells were read as positive. In addition, an optical density reading of <0.20 was considered negative, even if the reading was twofold greater than background, which generally ran well below 0.10.

The IgG subclass responses of the 20 infants and children to the RSV F and G glycoproteins are presented in Table 1. The antibody response to the F glycoprotein occurred predominantly in the IgG1 (100%) and the IgG3 (95%) subclasses, whereas only 15% of the patients had a rise in the IgG2 subclass response, and 10% of the patients had a rise in the IgG4 subclass response. The highest reciprocal mean log2 postinfection antibody titers were also in the IgG1 (10.5) and the IgG3 (9.6) subclasses. Among those responding to the F glycoprotein, the mean log2 fold antibody rises were 5.3 ± 0.4 (IgG1), 2.0 ± 0.0 (IgG2), 5.5 ± 0.6 (IgG3), and 7.0 ± 3.0 (IgG4). A similar pattern was seen for the G glycoprotein response. Most of these responses involved the IgG1 (85%) and IgG3 (95%) subclasses. Although 35% of the patients responded in the IgG2 subclass, the magnitude of the response was not significantly different from the response in the IgG2 subclass the F glycoprotein (Student's t test, >0.05). Only 5% of the patients responded to G glycoprotein with antibody in the IgG4 subclass. The highest reciprocal mean log2 postinfection antibody responses to the G glycoprotein were also in the IgG1 (7.0) and the IgG3 (8.5) subclass. Among those responding to the G glycoprotein, the mean log2 fold antibody rises were 3.5 ± 0.5 (IgG1), 4.3 ± 1.1 (IgG2), 5.0 ± 0.5 (IgG3), and 8.0 ± 0.0 (IgG4).

Previous studies, most of which used whole virions as antigen, showed that antiviral antibodies are mainly of the IgG1 and sometimes of the IgG3 subclass (1, 5, 6, 8–10, 15, 17). In contrast, the response to bacterial polysaccharide antigens appears to be primarily from the IgG2 subclass (16). Because of the known IgG subclass response to bacterial polysaccharides and the poor total IgG response to the G glycoprotein in the study by Ward et al. (20), it was reasonable to hypothesize that IgG2 constitutes the predominant response to viral and nonviral glycoproteins of the virus. However, the results of our study demonstrated that both glycoproteins F and G function as typical viral protein antigens, eliciting predominantly IgG1 and IgG3 antibody responses. In addition, we have shown that the antibody responses to another viral glycoprotein, the influenza A virus hemagglutinin, are also mainly of the IgG1 and IgG3 subclasses (D. K. Wagner, M. L. Clements, C. B. Reimer, M. Snyder, D. L. Nelson, and B. R. Murphy, unpublished observations).

The lack of an IgG2 response to the heavily glycosylated G protein could be simply a matter of the young age of the infants and children at the time of their primary RSV infection (12). We are evaluating the level of IgG subclass antibodies to the F and G glycoproteins in adult sera to see whether differences exist. Preliminary evidence suggests that adults have significantly more IgG2 antibody to the G glycoprotein than to the F glycoprotein, and the reciprocal is true for the IgG1 antibody levels (D. K. Wagner, D. L. Nelson, C. B. Reimer, E. E. Walsh, F. Henderson, and B. R. Murphy, unpublished observations).

LITERATURE CITED