The Response of Infants with Bronchiolitis to the Proteins of Respiratory Syncytial Virus

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SUMMARY

Acute phase sera were collected from 28 infants hospitalized with bronchiolitis due to respiratory syncytial (RS) virus and convalescent sera were collected from 24 of them. The sera were assayed for neutralizing antibodies by plaque inhibition, for antibodies to the viral proteins by Western blot against partially purified RS virus, and for their ability to inhibit attachment and fusion. Among the 28 acute phase sera, 27 had antibody to the attachment glycoprotein (G), 16 had antibody to the fusion glycoprotein (F), but none had antibody to the matrix protein (VPM). Both the geometric mean anti-G titre, and the geometric mean anti-F titre correlated with the 50% neutralizing dose (ND50) titre in the acute phase serum. Among the 24 convalescent sera, only four exhibited an increase in neutralizing antibody titre. The response to G appeared to be related to the acute phase ND50 titre. Of 17 infants with acute phase titres of less than 100 ND50/ml, 10 responded to G while there was no response to this protein in seven infants with acute phase titres greater than 100 ND50/ml. While only one infant responded to F, 18 responded to the phosphorylated nucleocapsid protein, VP32, and none responded to VPM. The ability of the acute phase sera to inhibit virus attachment to HeLa cells and to inhibit fusion correlated with the anti-G titre and the anti-F titre, respectively. However, there was no correlation between the inhibition of fusion and the anti-F titre in the convalescent sera, almost all of which inhibited fusion. These results suggest that the infected infants were responding to RS virus, but that their response to the viral proteins was either masked or slowed by residual maternal antibody. The inability to detect VPM in the acute and convalescent phase sera, as well as in 20 paired maternal and cord sera at a 1:50 dilution suggested that VPM, although it is one of the most prevalent viral proteins in both the virion and the infected cell, may be poorly antigenic in humans.

INTRODUCTION

Respiratory syncytial (RS) virus is a major cause of respiratory disease in infants under 1 year of age and remains an important pathogen up to the age of 2 (Gardner, 1968; Chanock & Parrott, 1965). Infection with this virus differs from other virus infections of childhood in that serious disease requiring hospitalization occurs primarily in the first 6 months of life when most infants are protected from the usual childhood viral infections by maternally derived antibody. Furthermore, an inactivated virus vaccine sensitized rather than protected the recipients, so that after natural exposure to the virus many of those who were vaccinated developed severe respiratory disease and required hospitalization. The mechanisms that produce both the unusual epidemiology of RS virus bronchiolitis and the sensitization by the inactivated RS virus vaccine are unknown, although it has been suggested that immune mechanisms are involved (for review, see McIntosh & Fishaut, 1980).

The RS virion contains seven polypeptides, three associated with the nucleocapsid, VPN, the phosphorylated VP32 and L, and four with the envelope, two glycoproteins (70K and 90K) and two unglycosylated proteins, the matrix protein, VPM (28K), and a 25K or 24K protein (Peeples...
& Levine, 1979; Pringle et al., 1981; Huang et al., 1985). The 70K protein is a proteolytically processed, disulphide-linked fusion protein (F) (Fernie & Gerin, 1982; Gruber & Levine, 1983, 1985b; Walsh & Hruska, 1983). The 90K attachment protein (G) is an unusual viral glycoprotein with oligosaccharides, mostly O-linked, contributing more than 50% to its Mr, and with an amino acid composition consisting of 30-6% serine and threonine and 10% proline (Gruber & Levine, 1985a; Fernie et al., 1985; Wertz et al., 1985; Levine et al., 1987).

Only recently has information about the immune response to the viral proteins become available. There appears to be a cytotoxic T cell response to VPN and F of RS virus (Bangham et al., 1986; Pemberton et al., 1987) and there are now four reports on the antibody response of humans to the RS virus proteins. With the exception of the study by Ward et al. (1983), most of the sera so far studied have come from infants older than 6 months, children and adults. In the first two studies, antibodies to the RS virus proteins were detected by radioimmunoprecipitation, in the third by ELISA and in the fourth by Western blotting.

In the first radioimmunoprecipitation study, the antigens were surface $^{125}$I-labelled RS virus-infected cells and $^{125}$I-labelled partially purified nucleocapsids (Ward et al., 1983). They found that maternal sera contained antibody to both glycoproteins and to VPN, with the lowest titre being to G, that none of the RS virus-infected infants developed antibodies to G and that infants older than 6 months responded to F and VPN, while infants under 3 months did not respond to either protein.

The antigen in the second immunoprecipitation study was $^{35}$S-methionine-labelled RS virus-infected cells (Vainionpaa et al., 1985). These workers also could not detect antibody to G in either the acute or convalescent phase sera of RS virus-infected children. Depending upon age at the time of infection, 4 months and older, there was either a weak or strong response to F and VPM.

The third study used an ELISA test to measure the antibody response in 18 infants with primary RS virus infection. These infants, who were 4 to 21 months old, included eight who were 4 to 8 months old. The antigens used were purified G and F and most of the infants developed a response to either one or both glycoproteins. Most of the older infants, 9 to 21 months, developed a neutralizing (N) antibody response, while most of the younger infants, 4 to 8 months, did not. Another difference between the two age groups was in the N and anti-G and anti-F titres which were eight- to 10-fold lower in the younger group (Murphy et al., 1986).

The most recent study utilized Western blotting with purified virus as antigen to detect antibodies to the viral proteins in 33 children and adults recovering from RS virus infection. All of these had antibodies to F and VPN, while 31 had antibodies to G and VPM, and 28 had antibodies to VP32 (Gimenez et al., 1987).

Because of the unusual amino acid composition of G and the variation in virus yield from batch to batch of infected cells, we used partially purified RS virus as antigen and Western blots to determine the concentration of antibody to the RS virus structural proteins in the acute and convalescent phase sera of infants with bronchiolitis.

**METHODS**

**Virus and cells.** The Long strain of RS virus was grown in monolayer cultures of HeLa cells (Levine & Hamilton, 1969).

$[^{14}$C]Glucosamine- and $[^{35}$S]methionine-labelled virus, which were used as markers, were grown and purified as previously described (Levine, 1977).

**Western blots.** The antigen was a partially purified RS virus prepared by two cycles of centrifugation through discontinuous sucrose gradients onto a 60% sucrose cushion, i.e. first through 20% sucrose in Hanks' balanced salt solution (HBSS) and the second through 30% sucrose in HBSS.

The antigen, at a concentration of 40 µg/0.5 cm well or nitrocellulose strip, was separated into its constituent proteins by SDS–PAGE under reducing conditions on 9% polyacrylamide gels. For the Western blots with infants' sera, the stacker gel was prepared with a well of 13 cm which was loaded with 520 µg of RS virus antigen. After SDS–PAGE, the separated viral proteins were transferred to a 15 cm × 15 cm nitrocellulose sheet. The conditions for SDS–PAGE and transfer were as previously described (Gruber & Levine, 1985a). All subsequent operations were at room temperature. To block the unreacted protein binding sites, the nitrocellulose sheet was shaken overnight, i.e. 18 to 24 h, on a horizontal shaker in a 5% solution of skim milk powder in phosphate-buffered saline.
RS virus infection in infants

Functional assays for antibody to viral proteins

Neutralization. The infants' sera were heated to 56 °C for 15 to 30 min to inactivate complement, and screened for N antibody at a 1:20, 1:100, 1:500 and 1:2500 dilution against approx. 50000 p.f.u./ml of RS virus. After 2 h incubation at room temperature, the virus-antibody mixture was diluted 1:100 and the surviving virus was assayed by plaque formation on HeLa cell monolayers (Levine & Hamilton, 1969). The 50% neutralizing dose (ND₅₀) was calculated by interpolation.

Fusion inhibition. The sera were tested for their effect on cell fusion in order to assay for their ability to inhibit a function of F. The method used bypassed the N activity of the serum. Monolayers of HeLa cells were infected with RS virus at an m.o.i. of approx. 3 p.f.u./cell. After attachment for 2 h at 37 °C, the inoculum was removed and the monolayers were overlaid with viral growth medium [minimum essential Eagle's medium with 5% inactivated foetal bovine serum (MEM 5% IFBS)] and incubated for an additional 3 h at 37 °C. The medium was then removed, the monolayers were washed and trypsinized and approx. 300 'infected' cells were plated together with about 300000 uninfected HeLa cells, in each well of a 24-well tissue culture cluster dish in a medium of either MEM with 2% of the infant serum being tested plus 3% IFBS or with the control medium, MEM 5% IFBS. Uninfected HeLa cells, about 300000/well, were also plated in MEM 5% IFBS to serve as a baseline control, since HeLa cultures always have a small number of multinucleated cells. Each sample was plated in triplicate for the assay. The cluster dishes were incubated for 48 h at 37 °C in a CO₂ tissue culture incubator. The overlay medium was removed, the monolayers were gently washed with PBS, fixed with methylene blue, and stained with methylene blue. The number of syncytia was counted under the microscope.

Attachment inhibition. The demonstration that G is responsible for RS virus attachment to susceptible cells (Levine et al., 1987) enabled us to set up an assay for anti-G function, i.e. to measure the effect of the infants' sera on the attachment of RS virus to HeLa cell monolayers. Purified [³⁵S]methionine-labelled virus was incubated overnight at 4 °C with a 1:50 dilution of the test serum in HBSS, or with either a 1:50 dilution of IFBS or 1% bovine serum albumin (BSA) in HBSS, as controls. For the virus-serum mixtures (approx. 3000 c.p.m./0.6 ml) were added to monolayers of approx. 3 x 10⁶ HeLa cells in 60 mm plastic tissue culture Petri dishes, 0.6 ml/dish, and these were incubated with continuous shaking on a reciprocal shaker (100 cycles/min) for 3 h at room temperature. The supernatant fluids were removed from the monolayers which were then extensively washed with cold HBSS. Both the supernatants and the monolayers were counted in a Packard Model 3255 Tri-Carb liquid scintillation counter. The monolayers were solubilized with 0.2 M-NaOH at 50 °C for 1 h and then neutralized with 0.2 M-HCl before mixing with the scintillation cocktail and counting. The supernatants were also brought to 0.2 M-NaOH, incubated at 50 °C and then neutralized.

Protein assay. The protein content of the partially purified viral antigen and the radiolabelled purified virus was determined by the method of Lowry et al. (1951).

Chemicals and radioisotopes. The BSA powder, fraction V, was purchased from Schwarz/Mann. The [¹²⁵I]-labelled Protein A (> 30 mCi/mg), [³⁵S]methionine (> 800 Ci/mmol) and [¹⁴C]glucosamine-HCl (2 to 5 Ci/mmol) were all purchased from Amersham.
RESULTS

Infants admitted to the Infectious Diseases Unit at the Children's Hospital of Michigan with the diagnosis of bronchiolitis in the winter of 1983 to 1984 were tested for the presence of RS virus in their nasopharyngeal secretions by the Virology Laboratory at the Children's Hospital. Both the acute phase serum, collected within 48 h after admission, and a convalescent phase serum, collected 2 to 4 weeks later, were tested for N antibody, attachment-inhibiting and fusion-inhibiting activities, and for antibodies to each of the viral structural proteins.

Thirty-four infants were hospitalized with bronchiolitis. Their average age was 4 months, with the youngest 20 days and the oldest 8 months. RS virus was isolated from 26 infants and an adenovirus from one. In addition, two of the infants, from whom no RS virus was isolated, were serologically diagnosed in this study as RS virus-infected. In one of these there was a greater than fourfold increase in the ND₅₀ titre, and in the other there was a fivefold or greater increase in the antibody titre, by Western blot, to three viral proteins, the two glycoproteins and VP32. Only the 28 infants whose disease was diagnosed as being caused by RS virus were included in this study. Convalescent serum was obtained from 24 of these 28 infants (22 infants from whom RS virus had been isolated and the two infants who converted serologically).

The antibodies to the RS virus proteins in the control human serum included in every Western blot are identified in Fig. 1. Our RS virus-positive control serum (lane 3) was reactive against G, F₁, VPN, VP32 and VP25 but not VPM or F₂ at this dilution of 1:50.

A typical result obtained with the Western blots of infants' sera is demonstrated in Fig. 2 which shows the immunoblots for four infants with bronchiolitis. Infant 1 (lanes 1 to 3 acute and 4 to 6 convalescent phase sera) showed a definite response to all the viral proteins, except for VPN to which the response was uncertain, and VPM, to which there was no response (since the lowest serum dilution tested was 1:50, it is uncertain that the 1:50 antibody titre to VPM in the convalescent serum represents a fourfold increase). Infants 2 (lanes 7 to 12) and 3 (lanes 13 to 18) responded only to VP32 (faint labelling of VP32 was visible on the X-ray film at a 1:250 dilution of the serum of infant 3), while infant 4 (lanes 19 to 24) responded to G and VP32 (the bands and blotches seen below VP25 in lanes 1, 4, 13, 16 and 22 are probably artefacts because they did not correspond to any of the viral structural proteins and were not consistently present).

Antibody to RS virus in the acute phase sera

Of the 28 acute phase sera, 12 had no detectable N antibody to RS virus, i.e. < 20 ND₅₀/ml, nine had titres between 20 and 100 ND₅₀/ml and seven had antibody titres greater than 100 ND₅₀/ml (Table 1). Contrary to the results obtained with radioimmunoprecipitation, 27 of the 28 infants had antibody to G. About 50% (10 of 21) of sera with N titres of less than 100 ND₅₀/ml, compared to about 86% (six of seven) of sera with titres greater than 100 ND₅₀/ml, had antibody to glycoprotein F₁.

The relationship between the geometric mean titres of the antibodies to G and F and the ND₅₀/ml are presented in Fig. 3. It appears that in the acute phase serum the N antibody titres correlate with both the anti-G titre and the anti-F titre, confirming the results reported by Murphy et al. (1986).

Antibody to RS virus in the convalescent phase sera

The response of the infants with bronchiolitis to the individual RS virus proteins is presented in Table 2. Among the 24 infants from whom a convalescent serum was obtained, only four responded with a fourfold or greater increase in the ND₅₀. The response to the glycoproteins appeared to be related to the ND₅₀ titre of the acute phase serum. Of the 17 infants with ND₅₀ titres of less than 100/ml, 10 responded with a fivefold or greater increase in the anti-G titre, while one responded to F. None of the infants with acute phase titres greater than 100 ND₅₀/ml responded to either glycoprotein. Eight infants responded to VPN, while none responded to VPM. Surprisingly, 18 of the 24 infants responded to VP32. As shown in Fig. 4 which compares the mean anti-RS titres in the acute and convalescent phase sera of the 24 infants, this response is probably a reflection of the low titre of the antibody to this protein in the acute phase serum rather than an indication that this antibody plays an important role in immunity to RS virus.
Fig. 1. Identification of the antibodies to the RS virus proteins present in the RS (+) adult human serum by Western blotting. Partially purified RS virus was separated into its constituent proteins by SDS-PAGE and Western-blotted. The nitrocellulose strip (lane 3) was then incubated in RS virus-positive control adult serum (1:50) (see Methods). Purified [14C]glucosamine- (lane 1) and [14C]-amino acid- (lane 2) labelled virus served as markers for the reactivity of control serum.

Fig. 2. Immunoblot of the acute and convalescent phase sera, from four infants with bronchiolitis, each at 1:50, 1:250 and 1:1250 dilutions. Lanes 1 to 6 infant 1: acute phase serum, lanes 1 to 3; convalescent phase serum, lanes 4 to 6. Lanes 7 to 12 infant 2: acute phase serum, lanes 7 to 9; convalescent phase serum, lanes 10 to 12. Lanes 13 to 18 infant 3: acute phase serum, lanes 13 to 15; convalescent phase serum, lanes 16 to 18. Lanes 19 to 24 infant 4: acute phase serum, lanes 19 to 21; convalescent phase serum, lanes 22 to 24. Lane 25, RS virus-positive control serum.
Fig. 3. Correlation between the neutralizing titre of the acute phase sera and the geometric mean titre of their antibodies to (a) F and (b) G.

Fig. 4. The geometric mean titres of the antibodies to RS virus and its proteins in the acute (□) and convalescent (□) sera of infants with bronchiolitis.

Table 1. Distribution of antibody to the RS virus glycoproteins in the acute phase sera from 28 infants with bronchiolitis due to RS virus, as a function of their RS virus-neutralizing antibody titre

<table>
<thead>
<tr>
<th>RS virus glycoprotein</th>
<th>ND_{50}/ml</th>
<th>&lt;20</th>
<th>20 to 100</th>
<th>&gt;100</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td></td>
<td>11/12*</td>
<td>9/9</td>
<td>7/7</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>5/12</td>
<td>5/9</td>
<td>6/7</td>
</tr>
</tbody>
</table>

* The numerator represents the number of antibody-positive sera and the denominator represents the total number of sera in that group.

Table 2. Relationship between the RS virus-neutralizing antibody titre in the acute phase serum and the response to RS virus in 24 infants with bronchiolitis

<table>
<thead>
<tr>
<th>Antibody</th>
<th>ND_{50}/ml in the acute phase serum</th>
<th>&lt;100</th>
<th>&gt;100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutralizing</td>
<td>Positive response</td>
<td>Negative response</td>
<td>Positive response</td>
</tr>
<tr>
<td></td>
<td>4*</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Anti-G</td>
<td>10</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Anti-F</td>
<td>1</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Anti-VPN</td>
<td>4</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Anti-VP32</td>
<td>14</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

* Number of infants.
RS virus infection in infants

However, the response to VP32 could indicate that the infants are also responding to the glycoproteins but that this response is masked by persisting maternal antibody. This is also indicated by the functional assays for the response to RS virus infection presented below.

Functional assays for the antibody response to RS virus infection

The acute and convalescent sera at a 1:50 dilution were screened for their ability to inhibit attachment and fusion. The results are presented in Fig. 5 and 6. In the acute phase sera there was a good correlation between the inhibition of fusion and the mean anti-F titre (Fig. 5). However, the correlation between the inhibition of fusion and the mean anti-F titre was not seen in the convalescent serum, i.e. almost all of the convalescent sera inhibited fusion, regardless of the titre. These data suggest that the infants were responding to F, but that we were not measuring this response in our Western blot assay. In the acute phase sera there was a good correlation between the inhibition of attachment and the mean anti-G titre (Fig. 6). However, since there was antibody to G in the acute phase serum of all infants from whom a convalescent serum was obtained, there was no inhibition of attachment in all of the acute phase sera. Therefore, it was only among the infants with acute phase anti-G titres of 50/ml that large changes in the percent inhibition of attachment could be observed. Of this group, the convalescent serum from three infants exhibited a threefold or greater increase in the percent inhibition of attachment. In two of these convalescent sera, the increased inhibition of attachment was accompanied by a fivefold or greater increase in the anti-G titre. The number of infants involved was too small to draw any valid conclusions.

Immunogenicity of VPM for humans

Since we found no antibodies to VPM in any of the acute or convalescent phase sera of the infants in this study or in our control adult human serum, we screened 20 paired maternal and cord sera to determine their antibody titres to the viral proteins, especially VPM. As expected, the titres of N antibodies and of antibodies to each of the viral proteins were the same for both sera of each pair. All 20 pairs had NDso titres of 100/ml or greater and all had significant levels of anti-G (> 250/ml). Only one pair did not have any detectable antibody to F at a 1:50 dilution, while the other 19 had anti-F titres ≥ 250/ml. The geometric mean N antibody titres and the geometric mean antibody titres to each of the viral proteins in the 20 pairs of maternal and cord sera are presented in Fig. 7. The lowest titres were to VP32 and VP25. Also, none was shown to have detectable antibody to VPM at a serum dilution of 1:50, which suggests that this protein may be less immunogenic than the other viral proteins in RS virus infections of humans.

DISCUSSION

The infants, all but five of whom were less than 6 months old, were hospitalized for bronchiolitis caused by RS virus. Both the acute and convalescent sera were assayed for their content of neutralizing antibody, attachment-inhibiting antibody (functional antibody to G), fusion-inhibiting antibody (functional antibody to F), and for antibodies to each of the virion glycoproteins. We assumed that, because of the infants' ages, the RS virus antibodies in the acute phase sera represented residual maternal antibody. We also assayed the RS virus antibody content of 20 paired maternal and cord sera in order to determine their RS virus antibody titres at birth.

Of 28 acute phase sera examined, 16 had detectable RS virus N activity, 27 had antibody to G and 16 had antibody to F. Also, in the acute phase sera the percent inhibition of viral attachment correlated with the geometric mean anti-G titre and the percent inhibition of fusion correlated with the geometric mean anti-F titre. Both the geometric mean anti-G and the geometric mean anti-F titres correlated with the N antibody titre as reported by Murphy et al. (1986). Antibody to VPM was not demonstrated in any of the acute phase sera.

Among the 24 infants from whom a convalescent serum was obtained, only four responded with an increase in the N titre, only one responded to F, none responded to VPM, 10 responded to G, while 18 responded to VP32. The response to G was related to the N antibody titre in the acute phase serum, since the 10 infants who responded had NDso titres of less than 100/ml,
Fig. 5. Relationship between the geometric mean anti-F antibody titre and the inhibition of fusion in the acute (●) and convalescent phase (○) sera of infants with bronchiolitis.

Fig. 6. Relationship between the geometric mean anti-G antibody titre and inhibition of attachment in the acute phase serum of infants with bronchiolitis.

Fig. 7. Geometric mean antibody titres to RS virus and its proteins in 20 paired maternal (□) and cord (□) sera.
while none of those infants with acute phase ND50 titres greater than 100/ml responded to G. These results suggest that the infants' response to the RS virus proteins were either masked or slowed by the residual maternal antibody. However, the response of the infants to VP32, as well as the breakdown in the correlation between the anti-F titre and the inhibition of fusion in the convalescent serum (Fig. 5), suggests that the infants were responding to RS virus infection. The poor N antibody response of the infants in this study was not surprising, since 20 of the 24 infants from whom a convalescent serum was obtained were younger than 6 months and 15 of the 24 were under 4 months. This age group has been reported to respond poorly to antigenic stimulation (Ross et al., 1971; Bruhn & Yeager, 1977; Murphy et al., 1986).

There are differences between our results and those reported previously, some of which may be attributable to the differences in the method of assay for antibodies, the antigen used and the ages of the populations studied. The differences in the prevalence of antibody to G in the acute phase sera of infants and in maternal sera observed in the two studies using the radioimmunoprecipitation assay (Ward et al., 1983; Vainionpaa et al., 1985) and in this study may be due to the unusual amino acid composition of this glycoprotein (Wertz et al., 1983): this results in it being poorly labelled with [35S]methionine (Pringle et al., 1981) and, with the possibility that it has a 'lower than average' complement of tyrosine, this would mean that its specific activity would be lower than that of F following 125I labelling. The difference between our results and those reported by Gimenez et al. (1987) (i.e. the greater response in their study to the viral proteins including VPM) could be due to the differences in the age of the populations studied. Since the youngest individual in their study was 3 years old, it is likely that this was not the infants' first exposure to RS virus and, therefore, that most of the patients in that study were undergoing secondary responses; we, however, were more likely dealing with primary responses. As in the study reported by Murphy et al. (1986), we were able to demonstrate a serum IgG response to G in some of the infants in this study, i.e. 10 of 24 infants. However, in contrast to that study, only one infant responded to F. The lower response observed in this study may be due to (i) the difference in the assays used, i.e. the greater sensitivity of the ELISA test used in their study, (ii) the difference in the antigens used, i.e. in the ELISA test the same quantity of each protein is used but the virus particle contains different quantities of G and F and (iii) the younger age of the infants in our study and, therefore, the higher levels of maternal antibody at the time of infection which were available to dampen the response. However, the advantage of using virus as antigen is that it is possible to observe the response to all the proteins.

One potential problem with the Western blot antibody assay is that this method does not preserve conformational epitopes and it is likely that such epitopes exist in all the structural proteins of RS virus. Conformational epitopes on the viral glycoproteins might be the stimulators of antibodies that neutralize virus or block attachment or fusion. Indeed, a conformational epitope that does not react in Western blots, non-conformational epitopes that react in blots with non-reduced but not with reduced protein and non-conformational epitopes that react in blots with reduced proteins have been described for F (Samson et al., 1986; Trudel et al., 1986; Walsh et al., 1986). Therefore, such epitopes could account for our inability to detect an increase in the anti-F titre, with the Western blot assay, in those convalescent sera with high anti-fusion activity but low anti-F titres. However, the relative importance of conformational and non-conformational epitopes has yet to be established. Although it is not possible to equate the results of different laboratories completely, there appear to be four or five epitopes on F, i.e. two or possibly three involved in neutralization and fusion, one involved in neutralization but not fusion, and one not involved in either function. Only one epitope, that which is not involved in either neutralization or fusion, is a conformational epitope whose reactivity is lost on denaturation by heating in SDS. The other epitopes react after denaturation, but the reactivities of two epitopes involved in neutralization and fusion and the epitope that is involved only in neutralization are lost after denaturation and reduction. However, the remaining epitope, involved in both neutralization and fusion, that reacts in Western blots after denaturation and reduction, appears to be a major or possibly the major epitope on F; a synthetic peptide, containing the sequence of a cleavage fragment of F1 that reacts with a monoclonal antibody to this epitope, is capable of blocking virus neutralization by this monoclonal antibody (Trudel et
al., 1986, 1987a, b; Walsh et al., 1986). Similarly, nine monoclonal antibodies to G, which included six that neutralized RS virus in the absence of complement, were bound to G in Western blots in which the viral proteins were transferred after reducing SDS–PAGE of purified RS virus (Tsutsumi et al., 1987). Therefore, although the loss of the correlation between the anti-F titre and the inhibition of fusion that we observed in the convalescent serum might have been due to the absence of the appropriate epitope(s) in the Western blot, it might also have resulted from the production by the infants of antibody, e.g. IgM or IgG3, that did not bind Protein A [IgG3 is apparently the subclass of IgG produced by very young infants with primary RS virus infection (Hornsleth et al., 1985)]. Indeed, the production of IgM and IgA, but not IgG, to F was demonstrated in infants younger than 6 months who were hospitalized with bronchiolitis caused by RS virus (Watt et al., 1986).

We also assayed the content of RS virus antibody in 20 paired maternal and cord sera. The highest titres in the paired maternal and cord sera and in the acute phase sera were to G, and no antibody to VPM could be demonstrated in these sera at the lowest dilution of serum tested, i.e. 1 : 50. These results are in general agreement with those reported for parainfluenza virus type 3 and measles virus, for which it was observed that there was a greater response to the attachment protein than to the fusion protein (Graves et al., 1984) and that there was a poor response to the M protein (Machamer et al., 1980; Norrby et al., 1981; Graves et al., 1984). Ward et al. (1983) also suggested that the M protein of RS virus was a poor immunogen for humans because antibodies to that protein were not found in normal adult sera.

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