Demonstration that Glycoprotein G Is the Attachment Protein of Respiratory Syncytial Virus

By S. LEVINE, R. KLAIBER-FRANCO AND P. R. PARADISO

1 Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, Michigan 48201 and 2 Praxis Biologics, 300 East River Road, Rochester, New York 14623, U.S.A.

(Accepted 20 May 1987)

SUMMARY

Two monospecific rabbit antisera to the G glycoprotein, one induced with purified G and the second with a recombinant vaccinia virus containing the gene for G, inhibit the attachment of purified [35S]methionine-labelled Long strain of respiratory syncytial virus to monolayers of HeLa cells. Attachment was not inhibited by monospecific rabbit antisera to glycoprotein F induced with either purified F or with a recombinant vaccinia virus containing the gene for F.

The envelope of respiratory syncytial (RS) virus contains two glycoproteins, F (M, 70K), a disulphide-linked glycoprotein and G (M, 90K) (Peeples & Levine, 1979; Fernie & Gerin, 1982; Gruber & Levine, 1983). The 70K protein has been identified as the fusion protein (Walsh & Hruska, 1983). G is an unusual viral glycoprotein with oligosaccharides, mostly O-linked, contributing approximately 60% to its Mr, and with a corresponding skewed amino acid composition (Gruber & Levine, 1985; Fernie et al., 1985; Wertz et al., 1985).

Monospecific antibody to G neutralizes viral infectivity (Walsh et al., 1984; Elango et al., 1986; Stott et al., 1986), and it has been assumed, by analogy with other Paramyxoviridae, that G is the viral attachment protein. However, there has been no direct demonstration that this is indeed so. We now report that monospecific anti-G serum, induced in rabbits with the G protein presented in different ways, does indeed inhibit viral attachment.

Two monospecific antisera to the G protein were tested. One was prepared by immunizing rabbits with G protein that had been purified by affinity chromatography on a column with a coupled monoclonal anti-G antibody according to the method of Walsh et al. (1984). It had an anti-RS virus titre of 2800 plaque reduction units 50 (PRU50)/ml and an anti-G titre of 3.7 × 105 units/ml when tested by ELISA against 20 ng of purified G per well. The second antiserum was from rabbits that were immunized with a recombinant vaccinia virus containing a cloned gene for G from the A2 strain of RS virus (Ball et al., 1986; Stott et al., 1986). It had an anti-RS virus titre of 64 PRU50/ml and an ELISA titre of 4 × 103 units/ml against a lysate of RS virus-infected cells (Stott et al., 1986). Control sera consisted of foetal bovine serum and monospecific serum produced in rabbits with F, purified on an anti-F monoclonal antibody column, or by immunizing rabbits with recombinant vaccinia virus containing the A2 RS virus gene for F (Wertz et al., 1987). The anti-RS virus titre of the serum from animals immunized with purified F was 10300 PRU50/ml and the anti-F titre was 6 × 106 ELISA units/ml against 20 ng purified F/well. The anti-RS virus titre of the serum from the vaccinated animals was 1024 PRU50/ml and the anti-F titre was 3 × 104 ELISA units/ml against a lysate of RS virus-infected cells (Wertz et al., 1987). The specificities of these sera against a partially purified RS virus are presented in the Western blot in Fig. 1. Both anti-G sera react with a second protein, M, 50K (Fig. 1, lanes 1 and 3). Because these two antisera were produced by such different methods, we
presume this protein to be either a breakdown product or precursor of G present in our partially purified antigen. A similar observation with a monoclonal antibody has been reported by Routledge et al. (1986).

We have previously determined the kinetics of RS virus attachment to HeLa cell monolayers at 37 °C and demonstrated that RS virus could also attach to monolayers at 4 °C (Levine & Hamilton, 1969). To restrict penetration and to limit the reaction to attachment, virus attachment was carried out at room temperature, and the results were confirmed at 4 °C.

The kinetics for the attachment of purified [35S]methionine-labelled virus to cell monolayers at room temperature was measured by both c.p.m. and p.f.u. (Fig. 2). Since the rates of attachment do not differ by more than twofold, we can use the binding of radiolabel as a measure of virus attachment in the presence of neutralizing antibody. The smaller fraction attaching when measured by c.p.m. is not due to saturation, since approximately the same fraction of
Short communication

Table 1. Effect of monospecific anti-G rabbit serum on RS virus attachment to monolayers of HeLa cells

<table>
<thead>
<tr>
<th>Serum</th>
<th>Dilution</th>
<th>Cell-associated c.p.m.</th>
<th>Supernatant c.p.m.</th>
<th>Cell + supernatant c.p.m.</th>
<th>Percent attached</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-F*</td>
<td>1:20</td>
<td>1403</td>
<td>2093</td>
<td>3496</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>1:60</td>
<td>2182</td>
<td>1665</td>
<td>3847</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>1:180</td>
<td>2647</td>
<td>1022</td>
<td>3669</td>
<td>77</td>
</tr>
<tr>
<td>Anti-G†</td>
<td>1:20</td>
<td>558</td>
<td>2918</td>
<td>3476</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>1:60</td>
<td>642</td>
<td>2867</td>
<td>3509</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>1:180</td>
<td>725</td>
<td>2840</td>
<td>3565</td>
<td>21</td>
</tr>
<tr>
<td>Anti-G‡</td>
<td>1:20</td>
<td>436</td>
<td>2532</td>
<td>2968</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>1:60</td>
<td>941</td>
<td>2477</td>
<td>3418</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>1:180</td>
<td>1621</td>
<td>2002</td>
<td>3623</td>
<td>47</td>
</tr>
<tr>
<td>IFBS§</td>
<td>1:20</td>
<td>1577</td>
<td>1450</td>
<td>3027</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>1:60</td>
<td>2047</td>
<td>1436</td>
<td>3483</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1:180</td>
<td>2192</td>
<td>1344</td>
<td>3536</td>
<td>64</td>
</tr>
<tr>
<td>1% BSA‖</td>
<td></td>
<td></td>
<td>1989</td>
<td>975</td>
<td>58</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td>3429</td>
<td>100</td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
<td></td>
<td>275</td>
<td></td>
</tr>
</tbody>
</table>

* Rabbits were immunized with purified F.
† Rabbits were immunized with purified G.
‡ Rabbits were immunized with a recombinant vaccinia virus containing the gene for G.
§ Foetal bovine serum inactivated at 50 °C for 30 min.
‖ Bovine serum albumin, fraction V.

radiolabel attached when as little as 100 c.p.m. to as much as 100000 c.p.m. of purified \[^{35}\text{S}\]methionine-labelled virus was added to the cell monolayers (data not shown). Similar kinetics were also observed at 4 °C (data not shown).

To determine which monospecific antibody would inhibit RS virus attachment, purified \[^{35}\text{S}\]methionine-labelled Long strain of RS virus (Levine, 1977), with p.f.u. to c.p.m. ratios of either 110 or 30, was incubated overnight at 4 °C with various dilutions of the monospecific or foetal bovine serum in Hank’s balanced salt solution (HBSS), or 1% bovine serum albumin. The virus–antibody mixtures, containing approximately 3000 c.p.m./0.6 ml, were then added to monolayers of 3 × 10^6 HeLa cells in 60 mm tissue culture plastic Petri dishes (0.6 ml/dish) and the dishes were incubated with continuous shaking on a reciprocal shaker, for 3 h at room temperature. Then the supernatant fluid was removed from the monolayers which were then washed several times with cold HBSS. Both the supernatant and the monolayers were counted in a Packard Model 3255 Tri-Carb liquid scintillation counter. For counting, the cell monolayer was made soluble with 0.2 M-NaOH at 50 °C for 1 h and then neutralized with 0.2 M-HCl before mixing with a scintillation cocktail.

An example of an attachment experiment is presented in Table 1. In this experiment the standard deviation of the average of the sum of the residual and cell-associated c.p.m. was approx. 8% and, within a single experiment, this standard deviation was always 10% or less. With the exception of the slight decrease in the percentage of \[^{35}\text{S}\]methionine-labelled RS virus attached at the 1:20 dilution of the foetal bovine serum and the anti-F serum, only the anti-G sera inhibited the attachment of RS virus to HeLa cell monolayers. The same results were obtained in four additional experiments at room temperature, and similar results were obtained at 4 °C (data not shown).

Despite the greater concentration of antibody to F than to G in the sera produced by both methods of immunization, only the antisera to G inhibited attachment. By contrast, anti-F, but not anti-G, blocked fusion, confirming the conclusion of Walsh & Hruska (1983) that F is the fusion protein (data not shown). Thus, as is the case with all Paramyxoviridae, the fusion and attachment functions of RS virus are separated and assigned to different glycoproteins.
Short communication

This work was supported by grants to S.L. from the Thrasher Research Fund (no. 2797) and WHO (V21/181). We thank Dr G.W. Wertz, of the Department of Microbiology of the School of Medicine of the University of Alabama, Birmingham, Ala., U.S.A. for her generous gift of the rabbit antisera produced with the recombinant vaccinia viruses.

REFERENCES


(Received 25 March 1987)