Antigenic and immunogenic analysis of group A and group B respiratory syncytial virus G proteins expressed from recombinant baculoviruses

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The attachment glycoprotein G plays a major role in the antigenic variability of respiratory syncytial (RS) virus. We have expressed from recombinant baculoviruses antigenic group A and group B RS virus G proteins (designated bacAG for the group A and bacBG for the group B virus G protein). The insect cell-produced G proteins migrated more rapidly in SDS–PAGE as compared to HEp-2 cell-derived G proteins owing to glycosylation differences. Antigenicity was tested by immunofluorescence; five of five group cross-reactive, five of six group A-specific, and six of six group B-specific MAbs reacted appropriately with bacAG and/or bacBG. In addition, bacAG and bacBG reacted with human polyclonal antibodies to RS virus. Cotton rats were immunized with bacAG, bacBG or a control lysate and challenged intranasally with a group A RS virus. The bacAG-immunized group had a statistically significant reduction in viral replication in the lungs (lung titres as mean \( \log_{10} \) p.f.u./g ± SD, bacAG = 3.1 ± 1.2; control = 4.8 ± 0.6, \( P = 0.013 \)). The bacBG-immunized group showed less reduction in viral titres (bacBG lung titres = 4.1 ± 0.6, \( P = 0.13 \) for bacBG compared to control). Thus, as expected, homologous protein (bacAG) immunization provided more protection against viral replication than immunization with the heterologous protein (bacBG). The G protein of RS virus expressed in insect cells had antigenic and immunogenic features which were similar to that of the G protein expressed in mammalian cells. The baculovirus-expressed G proteins should be useful for the study of immune responses to RS viruses.

Introduction

Respiratory syncytial (RS) virus is the viral agent most frequently isolated from children with acute respiratory infections in developing and developed countries (McIntosh & Chanock, 1990). At the present, no safe and effective vaccine is available. One of the unexplained issues in RS virus immunobiology is the ability of the RS viruses to avoid the host immune response. Examples of this include the infection of infants in the presence of transplacentally acquired antibodies and the occurrence of re-infections throughout life (Glezen et al., 1981; Henderson et al., 1979). Antigenic variability among RS viruses appears to contribute to this feature of RS viruses (Mufson et al., 1987; Waris, 1991).

RS virus is a member of the genus Pneumovirus in the family Paramyxoviridae. The attachment protein G of RS virus, an important target of the host immune response, is a type II integral membrane protein. The G protein is heavily glycosylated and 50% of the molecular weight of the mature protein (\( M_r, 90000 \)) may be provided by carbohydrates, predominantly O-linked with some N-linked sugars. In keeping with this degree of glycosylation, the G protein has a 30% content of serine and threonine, which are potential sites for O-linked sugars. Thus the 298 amino acid, \( M_r, 32587 \) protein encoded by the A2 RS virus G gene is modified by carbohydrate addition to obtain the mature \( M_r, 90000 \) form (Wertz et al., 1985, 1989).

There are two major antigenic groups (A and B) of RS virus (Anderson et al., 1985; Mufson et al., 1985). The greatest antigenic and sequence differences between the two groups are in the G protein (Anderson et al., 1985; Johnson et al., 1987a, b; Mufson et al., 1985; Stott et al., 1987; Sullender et al., 1990). Children initially infected with a virus of one antigenic group are more likely to experience re-infection with a virus of the heterologous as compared to the homologous antigenic group (Mufson et al., 1987; Waris, 1991). The antibody responses of children after infection with a virus of known antigenic group are higher to the homologous as compared to the heterologous G protein and neutralizing
Table 1. Immunoﬂuorescence reactivity of G protein-speciﬁc MAbs with G proteins expressed in insect cells

<table>
<thead>
<tr>
<th>Reactivity</th>
<th>MAb</th>
<th>Group A</th>
<th>Group B</th>
<th>bacAG</th>
<th>bacBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A and B</td>
<td>131–2g</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>221/1G</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>130–5f</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>29</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>L9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Group A speciﬁc</td>
<td>021/2G</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>63G</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>78G</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>63–10f</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>130–6d</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>130–2g</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Group B speciﬁc</td>
<td>22</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>-</td>
<td>+</td>
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<td>26</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>C1</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C8</td>
<td>-</td>
<td>+</td>
<td>+</td>
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antibody titres are also higher to a homologous as compared to a heterologous group virus (Hendry et al., 1988).

RS virus proteins, either purified from RS virus-infected cell lysates by immunoaffinity chromatography or expressed from vaccinia virus recombinants, have been used to investigate the role of the individual proteins in the immune response (Johnson et al., 1987 a; Stott et al., 1987; Walsh et al., 1987). One limitation of the immunoaffinity-purified proteins is their potential for contamination with other RS virus proteins (Walsh et al., 1987). The vaccinia virus recombinants have overcome this limitation and are particularly useful when expression from a replicating virus is desired. However, at times antigenic and immunogenic studies need to use individual proteins which are not being actively expressed from a replicating virus. The baculovirus system provides a method for the production of such proteins. We have undertaken the cloning into and expression from recombinant baculoviruses of the G proteins of group A and group B RS viruses. Here we describe an antigenic and immunogenic characterization of these proteins.

These studies allowed us to determine whether the glycosylation differences which occur between insect and mammalian cells were antigenically important for the heavily glycosylated G protein. The recombinant baculoviruses described here should provide a source of RS virus G protein for studies of RS virus immune responses.

Methods

Cells and viruses. The prototype group A (A2 strain) and B (8/60 strain) RS viruses were cultivated in HEp-2 cells grown in Medium 199 (Gibco/BRL) supplemented with 5% fetal calf serum (FCS) with penicillin, kanamycin and streptomycin (PKS). A recombinant baculovirus expressing the cytomegalovirus glycoprotein B (bac3.1) was previously described (Wells et al., 1990). Wild-type (Autographa californica multinucleocapsid nuclear polyhedrosis virus, AcMNPV) and recombinant baculoviruses were cultivated in the following insect cell lines. Spodoptera frugiperda (Sf9) cells were maintained in Grace’s medium (Gibco/BRL) supplemented with 10% FCS and PKS. A cell line derived from Triechophtis ni eggs, Tn 5Bl, (Wickham et al., 1992) was obtained commercially (High 5 cells; Invitrogen) and maintained in Excell 401 medium (JRH Biosciences) with PKS.

Plasmids and recombinant baculovirus construction. The plasmid containing the group A (strain A2) RS virus G protein cDNA was
generously provided by Gail Wertz (University of Alabama, Birmingham) (Ball et al., 1986). The group B (strain 8/60) G protein cDNA was described previously (Sullender et al., 1990). The cDNAs were moved into plasmids pAC-YM1 (for the group A clone) or pvl1392 (for the group B clone) to place the cDNA downstream of the polyhedrin promoter using standard techniques (Sambrook et al., 1989). Transfer of the cDNAs into wild-type baculoviruses was performed using the Baculogold system (Pharmingen). Positive clones were identified by immunofluorescence of infected cells with RS virus G protein-specific monoclonal antibodies and were subjected to three rounds of plaque purification. Viral stocks were prepared on SF9 cells. The group A and B RS virus G protein-expressing recombinants were designated bacAG and bacBG, respectively.

**Immunoblots.** Tn 5Bl-4 cells infected with baculoviruses were lysed in buffer consisting of 25 mM-Tris-HCl pH 8.0, 0.15 M-NaCl, 1% Triton X-100, 1% deoxycholic acid, 0.2% SDS and 8 M-urea and clarified by centrifugation. HEp-2 cells (uninfected or infected with RS virus) were lysed in a similar buffer except that it lacked SDS and urea, clarified by centrifugation, and the resulting lysate was made 0.2% with regard to SDS. The following protease inhibitors were included in the lysis buffers: 0.1 mM-AEBSF [4-(2-aminoethyl)-benzenesulphonylfluoride], 0.5 µg/ml leupeptin I, 1 µg/ml pepstatin, 1 µg/ml E64, 0.1 mM-EDTA. Immunoblotting was performed as previously described (Sullender, 1995). The effects of the metabolic inhibitors tunicamycin and monensin on G protein synthesis were also analysed by immunoblotting. For these experiments, Tn 5Bl-4 cells were infected with bacAG and grown in the presence of either tunicamycin (5 µg/ml) or monensin (0.05 µm). HEp-2 cells were infected with the A2 strain of RS virus and grown in the presence of either tunicamycin (10 µg/ml) or monensin (5 µm). Cell lysates were harvested after a 1 day infection.

**Immunofluorescence.** Infected cells were taken up, washed with PBS, applied to multilwell glass slides and fixed in acetone. Immunofluorescence was performed as previously described (Sullender, 1995). Each assay included wild-type baculovirus-infected cells or uninfected HEp-2 cells as a negative control. A positive result was assigned when there were multiple fluorescent cells which in number and in fluorescence intensity clearly differed from the negative control cells. Cell surface immunofluorescence was performed in a similar manner except that cells were grown on glass coverslips and fixed with 0.5% paraformaldehyde after incubation with the monoclonal antibody.

**Antibodies.** Monoclonal antibodies were generously provided by several investigators (see Table 1). Human serum or plasma samples which had been obtained from adults for other purposes were used as sources of human antibodies to RS virus. In order to decrease the background reactivity of the human polyctonal antibody samples to the cotton rats antibodies overnight at 4 °C, then removed by centrifugation. powder from Tn 5Bl-4 cells was incubated with diluted human

**Results**

**Expression of RS virus G proteins from recombinant baculoviruses**

Recombinant baculoviruses bacAG and bacBG were tentatively identified by immunofluorescence as described in Methods. Protein expression in insect cells infected with bacAG and bacBG was assessed by immunoblotting infected cell lysates (Fig. 1). The recombinant baculovirus-expressed G proteins were compared to the G proteins produced in HEp-2 cells infected with prototype group A (A2 strain) or group B (8/60 strain) RS viruses. The mature G proteins from mammalian cells (GrsA and GrsB, Fig. 1, lanes 1 and 3) migrated more slowly than the mature G protein produced in insect cells (bacAG and bacBG, Fig. 1, lanes 2 and 4). As previously described, the A2 RS virus G protein (GrsA) migrated more slowly than the 8/60 RS virus titre (p.f.u./g lung tissue) was measured by plaque assay on Vero cells. Serum antibodies against RS virus were measured by ELISA (Sullender et al., 1990). Mean lung virus titres and ELISA antibody titres were compared using unpaired two-tailed Student's t tests.

![Fig. 1. Immunoblot analysis of RS virus- and baculovirus-expressed G proteins.](image-url)
Characterization of insect cell expressed G proteins

In order to better understand the mobility differences observed among the insect cell- and mammalian cell-produced proteins, the forms of the G protein produced in the presence of glycosylation inhibitors were analysed (Fig. 2). In this experiment, as was also seen in Fig. 1, the mature forms of G protein produced in mammalian and insect cells in the absence of inhibitors differed in mobility (lanes 1 and 2, GrsA and bacAG, Fig. 2). In addition, from both cell lines forms of the G protein which migrated near the 43 kDa standard were visible, as two faint bands from the mammalian cells and a broad band from the insect cells. These forms (G45, Fig. 2) had a mobility which was similar to that of previously described precursor forms of the G protein with attached N-linked sugars (high mannose, immature complex) which migrate near 45 kDa (Collins & Mottet, 1992; Lambert, 1988; Wertz et al., 1989).

In the presence of tunicamycin, which inhibits the addition of N-linked oligosaccharides, the mammalian cell G protein (Fig. 2, lane 3) migrated more rapidly than the mammalian cell G protein produced in the absence of tunicamycin (Fig. 2, lane 1) (Gruber & Levine, 1985). Tunicamycin had a more dramatic inhibitory effect on the insect cell-produced G protein, with the predominant species migrating between the 43 kDa and 29 kDa standards (Fig. 2, lane 4). This mobility was compatible with that of the unglycosylated precursor form of G (Gp), which migrates in SDS-PAGE with an apparent molecular mass of 36 kDa (Collins et al., 1984; Lambert, 1988; Wertz et al., 1989). Although this might have suggested there were only N-linked sugars added to the G protein in insect cells, experiments with monensin (see below), indicated that O-linked sugars were also added to the insect cell-expressed G protein. The concentration of tunicamycin used in this experiment may have interfered with protein transport and thus resulted in impaired glycosylation of the protein (Elbein, 1987). In another experiment (not shown) forms of the G protein were detected which were less mobile than the G45 forms but were more mobile than the mature product. These forms likely represented G protein with only O-linked sugars added. After tunicamycin treatment, no bands were seen in either the insect or mammalian cell lanes which appeared to be the G45 form of the G protein with N-linked sugars attached.

With monensin treatment, which impairs the migration of proteins through the Golgi complex and thus inhibits the addition of O-linked oligosaccharides, there was a diminution in detection of mature products, and an increase in forms migrating near the 43 kDa standard (Fig. 2, lanes 5 and 6) (Gruber & Levine, 1985). These forms had a mobility similar to that of the presumed N-linked sugar forms seen also with G proteins produced in the absence of inhibitors (Fig. 2, lanes 1 and 2, G45). The mammalian cell-expressed G protein forms which migrated near 43 kDa appeared to migrate slightly more slowly than the forms found in the insect cells. With the combination of tunicamycin and monensin both the cell types produced G proteins which migrated between the 43 kDa and 29 kDa standards, as expected for the 36 kDa Gp forms (Fig. 2, lanes 7 and 8). The presumed Gp forms of the G proteins from mammalian and insect cells showed identical mobility, the G45 forms differed slightly in mobility and the mature products differed more in mobility. Thus, the baculovirus-expressed G proteins appeared to have both
Table 2. Analysis of protection against viral challenge in cotton rats immunized with baculovirus-expressed RS virus G protein

Viral titres in the lungs were measured by plaque assay and are shown as mean ± SD. Antibody titres were measured by ELISA from samples obtained at the time of lung harvest and are shown as the reciprocal of the titre (log_{10}), mean ± SD. Antibody titres were performed for two animals in each group pre-challenge and for all the animals in each group post-challenge. Differences were measured by Student's t test, for the differences in lung titres $P = 0.013$ for bac3.1 compared with bacAG and $P = 0.01$ for bac3.1 compared with bacBG. For the differences in ELISA titres between the bacAG compared with bacBG groups, pre-challenge for the A2 antigen $P = 0.16$ and for the 8/60 antigen $P = 0.10$ and post-challenge for the A2 antigen $P = 0.18$ and for the 8/60 antigen $P = 0.01$.

<table>
<thead>
<tr>
<th>Immunogen (no. of rats)</th>
<th>Lung viral titres (log_{10} p.f.u./g)</th>
<th>Antibody to A2 pre-challenge</th>
<th>Antibody to 8/60 pre-challenge</th>
<th>Antibody to A2 post-challenge</th>
<th>Antibody to 8/60 post-challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>bac3.1 (6)</td>
<td>4.8 ± 0.6</td>
<td>&lt; 1.5 ± 0</td>
<td>&lt; 1.5 ± 0</td>
<td>&lt; 1.5 ± 0</td>
<td>&lt; 1.5 ± 0</td>
</tr>
<tr>
<td>bacAG (6)</td>
<td>3.1 ± 1.2</td>
<td>3.1 ± 0.5</td>
<td>2.5 ± 0.5</td>
<td>3.6 ± 0.5</td>
<td>2.7 ± 1</td>
</tr>
<tr>
<td>bacBG (5)</td>
<td>4.1 ± 0.6</td>
<td>2.2 ± 0.3</td>
<td>3.8 ± 0.4</td>
<td>3.2 ± 0.4</td>
<td>4.2 ± 0.2</td>
</tr>
</tbody>
</table>

N- and O-linked sugars added. The differences in mobility seemed likely to be due to differences in the carbohydrates added, as previously described for a chimeric FG protein expressed in insect as compared to mammalian cell lines (Wathen et al., 1991). No formal estimation of relative mobility was performed because the protein size standards used in these immunoblots were pre-stained and produced relatively broad bands which do not migrate exactly according to the size of the unstained proteins (product information, Gibco/BRL).

Cell surface immunofluorescence

The attachment protein G expressed in mammalian cells appears on the surface of the cells (Wertz et al., 1989). We sought to determine whether the G protein would also appear on the surface of insect cells infected with the recombinant baculoviruses expressing the G protein. Both bacAG- and bacBG-infected insect cells demonstrated cell surface immunofluorescence (not shown). Thus, even though there were differences in glycosylation between the insect cell- and mammalian cell-expressed proteins, the insect cell-expressed proteins were transported to the cell surface.

Antigenic analysis

We assessed the extent to which the insect cell-produced G protein might differ antigenically from the mammalian cell-derived G protein. The reactivities of bacAG and bacBG with MAbs were assessed by indirect immunofluorescence (not shown) and the results were summarized (Table 1).

Both group cross-reactive and group-specific MAbs were tested (references in Table 1). All five of the group cross-reactive MAbs reacted with both bacAG and bacBG. Among the six group A-specific MAbs, five were reactive. One group A-specific MAb (78G) failed to react (García-Barreño et al., 1989). MAb 78G was the only MAb we used as hybridoma cell culture supernatant rather than as ascitic fluid. All six of the group B-specific MAbs reacted with the G protein expressed from bacBG. Thus testing with MAbs from several different laboratories revealed differences in reactivity for only one MAb among the mammalian cell- and insect cell-derived proteins. The tested MAbs included antibodies known to recognize different antigenic sites and/or epitopes and which depend on the presence of different regions of the G proteins for reactivity (Table 1) (Sullender, 1995).

In order to determine whether human antibodies would recognize the baculovirus-expressed G proteins we tested samples from six adults by immunofluorescence against bacAG, bacBG and wild-type baculovirus-infected Tn 5B1-4 cells and against RS virus-infected or uninfected HEP-2 cells. All six samples reacted with bacAG, bacBG and with mammalian cells infected with either A2 or 8/60 RS virus, but not with control insect or mammalian cells (not shown). Thus, the polyclonal response of humans to RS virus included antibodies which reacted with the baculovirus-expressed G proteins.

Animal protection

The immunogenicity of bacAG and bacBG was investigated by immunizing cotton rats with bacAG, bacBG or a control baculovirus cell lysate (bac3.1, expressing human cytomegalovirus glycoprotein B), challenging the rats with live RS virus, and measuring the extent to which viral replication in the lungs was decreased (Table 2).

After challenge with a group A strain RS virus, the rats immunized with bacAG had a significant reduction in viral titres in the lungs as compared to the control animals (a 1.7 log_{10} reduction for bacAG as compared to bac 3.1, $P = 0.013$ for bacAG as compared to bac3.1 lung titres). Two of six rats in the bacAG group had no virus recovered from their lungs, while all the other animals had measurable virus titres in the lung. As expected, the bacBG group had a reduction in lung titres which was less than that observed for bacAG and which...
did not achieve statistical significance (a 0.7 log$_{10}$ difference as compared to bac3.1, P 0.1) (Johnson et al., 1987 a; Stott et al., 1987; Sullender et al., 1990). The bacAG group represents a homologous challenge with a group A strain virus, as compared to the heterologous challenge for the bacBG group.

Serum antibodies were measured by ELISA with A2 or 8/60 RS virus-infected cell lysates. Nine days after the second vaccine dose and before viral challenge, two animals from each group were bled and the presence of antibodies to RS virus was determined. As shown (Table 2), immunization with bacAG and bacBG, but not with bac3.1, stimulated the production of antibodies which reacted specifically with the RS virus antigens. Higher antibody titres were measured to the homologous as compared to the heterologous antigens and although these results were determined from only two samples from each group, they were in agreement with the pattern of protection against viral replication described above for the lungs. Antibody titres were also determined from samples obtained after viral challenge at the time of lung harvest. Interestingly, for the ELISA done with the A2 antigen there were not significant differences between the bacAG and bacBG groups. For the 8/60 antigen ELISA, the bacBG groups had significantly higher titres as compared to the bacAG group. The limited differences in titres among the A2 ELISA results and the greater differences in the 8/60 ELISA results appeared to be due to the boosting effect of the A2 virus challenge after heterologous immunization.

These data show that the baculovirus-expressed G proteins of RS virus when used as immunogens were capable of stimulating an RS virus-specific antibody response. In addition, upon challenge with RS virus a significant resistance to viral replication in the lungs was demonstrated.

Discussion

In this report we describe the expression from recombinant baculoviruses of the G proteins of a group A and a group B RS virus. Differences in mobility in SDS-PAGE among the insect cell- and mammalian cell-expressed G proteins appeared to be due to differences in glycosylation. The baculovirus-expressed G proteins were found to maintain a broad reactivity with both G protein-specific MAbs and polyclonal antibodies to the G protein, indicating antigenic similarities with the mammalian cell-derived G proteins. In addition, there were immunogenic similarities, with bacAG immunization providing significant protection against challenge with live RS virus. Thus, the baculovirus-expressed G proteins had antigenic and immunogenic similarities to the G proteins of RS virus produced in mammalian cells.

The G protein of RS virus is an important target of the host immune response. In addition, the G protein plays a major role in the antigenic and genetic differences which occur both between and within the two major antigenic groups of RS virus (Sullender & Wertz, 1991). Investigations of RS virus pathogenesis would be facilitated by access to adequate quantities of individual RS virus proteins such as the G protein. The baculovirus system offers the opportunity to produce G proteins for antigenic and immunogenic studies with no possibility of contamination with other RS virus proteins.

The attachment protein G of RS virus is extensively glycosylated, including predominantly O-linked with some N-linked sugars (Wertz et al., 1985, 1989). The G proteins expressed from recombinant baculovirus-infected cells migrated more rapidly in SDS-PAGE than the G proteins expressed in RS virus-infected mammalian cells (Fig. 1). Comparisons of the forms produced in the presence of inhibitors of glycosylation showed the presumed unglycosylated precursor (Gp) forms to have identical mobilities among the insect cell- and mammalian cell-derived proteins (Fig. 2). Thus, the observed mobility differences for the mature G proteins from the two cell lines were likely due to differences in glycosylation. The data also suggested that there were both N- and O-linked sugars added to insect cell-expressed protein (Fig. 2). One caveat to the interpretation of these results is the variable effects that sugars can have on the migration of glycoproteins in SDS-PAGE.

A chimeric protein (FG) composed of part of the fusion protein (F) and the extracellular domain of the G protein has been expressed from recombinant baculoviruses (Wathen et al., 1989). The FG protein expressed in Sf9 cells contains two types of O-linked oligosaccharides, GalNAc and Galβ1-3GalNAc, with none containing sialic acid. Only one form of N-linked oligosaccharide, a fucosylated trimannosyl core structure, occurs. The FG protein expressed in mammalian cells migrates more slowly than insect cell-expressed FG protein (Wathen et al., 1991). Glycoproteins expressed in insect cells are frequently smaller than the same glycoproteins expressed in mammalian cells, owing to differences in glycosylation (Jarvis & Summers, 1992). For the FG protein, and likely for the baculovirus-expressed G proteins described here, these differences in mobility were due to the presence of truncated oligosaccharide side chains and the lack of sialic acid (Wathen et al., 1991).

As part of our characterization of the baculovirus-expressed G proteins we showed that these proteins were present on the surface of infected insect cells. This demonstrated that the insect cell-expressed G proteins were competent for folding and transport to the cell surface. Studies of mammalian cell-expressed G proteins
have shown that even G protein with no sugars attached reaches the cell surface, albeit at reduced levels (Lambert, 1988; Wertz et al., 1989).

Given the differences in attached sugars among the mammalian cell- and insect cell-derived G proteins, we asked what were the effects on the antigenic reactivity of the G protein? We assessed antigenic reactivity using both MAbs and polyclonal antibodies. All of the group cross-reactive, five of six group A-specific and all of the group B-specific MAbs were reactive with the appropriate baculovirus-expressed G proteins. The one group A-specific MAb (78G) which did not react with bacAG was tested only as a cell culture supernatant and the fact that it was used in this relatively dilute state may have had implications for its reactivity. However, it is interesting that MAb 78G does not react with unglycosylated G protein (Palomo et al., 1991). In addition to the broad reactivity found among MAbs, the RS virus antibodies found in adult humans were demonstrated to react with the baculovirus-expressed G proteins by immunofluorescence.

Thus, testing with MAbs and with polyclonal antibodies demonstrated antigenic similarities among the insect cell-derived as compared to the mammalian cell-derived G proteins. The presence of sugars, particularly O-linked sugars, is important for the reactivity of many G protein-specific MAbs (Palomo et al., 1991). Therefore, the glycosylation differences among the mammalian cell- and insect cell-expressed G proteins were potentially quite important. Testing with a wider variety of G protein-specific MAbs might have revealed additional antigenic differences. The G protein has also been expressed in a prokaryotic system, the unglycosylated G protein was recognized by polyclonal and neutralizing MAbs to the G protein and stimulated a neutralizing antibody response when used as an immunogen (Martin-Gallardo et al., 1993). Thus, the presence of sugars may not be necessary for the maintenance of all the potential neutralizing epitopes of the G protein (Martin-Gallardo et al., 1993; Norrby et al., 1987).

The immunogenicity of the baculovirus-expressed G proteins was also assessed (Table 2). Immunization with bacAG reduced viral replication in the lungs after viral challenge. BacBG immunization decreased the lung titres less, as would be expected after challenge by a virus heterologous to the immunizing G protein (Johnson et al., 1987a; Stott et al., 1987; Sullender et al., 1990). These results were in agreement with previous studies of G protein immunogenicity. Vaccinia virus recombinants expressing the G protein provide cotton rats with a significant protection against challenge by live RS virus (Olmsted et al., 1986; Stott et al., 1986), as does immunoaffinity-purified G protein (Walsh et al., 1987). Thus, although not compared directly in these experiments, the baculovirus-expressed G proteins appeared to have immunogenic similarities to the mammalian cell-expressed G proteins.

We have described the expression from recombinant baculoviruses of the glycoprotein G of RS viruses of both antigenic groups A and B. Glycosylation differences were found between the mammalian cell- and insect cell-expressed G proteins. However, antigenically and immunogenically the G proteins from mammalian and insect cells were shown to have similarities. The baculovirus-expressed G proteins should be useful for the study of immune responses to RS virus.

The A2 strain RS virus G protein cDNA and the cotton rats used in these studies were generously provided by Gail Wertz (Birmingham, Alabama, USA). RS virus-specific MAbs were kindly supplied by L. Anderson (Atlanta, Georgia, USA), J. Melero (Madrid, Spain), G. Taylor (Compton, UK) and E. Walsh (Rochester, New York, USA). We thank Dana Pinson for manuscript preparation and Jennifer Gross and April Johnson for technical assistance. Support was received from Public Health Service grants AI13425 and AI37197 (WMS). Support for the maintenance of the cotton rat colony was received from AI20181 (Gail Wertz). This work was presented in part at the Society for Pediatric Research, Seattle WA, May 2–5, 1994; the American Society for Virology 14th Annual Meeting, Austin, Texas, July 8–12, 1995; and the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, California, September 17–20, 1995.

References


