Protection of Cotton Rats against Human Respiratory Syncytial Virus by Vaccination with a Novel Chimeric FG Glycoprotein

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SUMMARY

The cotton rat model of experimental human respiratory syncytial virus (RSV) infection was used to study the efficacy of FG, a novel chimeric glycoprotein which was expressed in insect cells using a baculovirus vector. FG contained the extracellular regions of the F (fusion) and G (attachment) glycoproteins of RSV. Vaccination with FG resulted in induction of neutralizing antibody and was correlated with protection of lung tissue from RSV challenge against both serogroup A and B virus strains. Both crude FG taken from supernatants of insect cells and affinity-purified FG were immunogenic and active against RSV. FG vaccination was effective by three routes of administration, following a single dose, and when administered with different adjuvants.

INTRODUCTION

Human respiratory syncytial virus (RSV), a paramyxovirus, is the major cause of severe lower respiratory disease in children under 1 year of age (Parrott et al., 1973). Previous attempts to produce a safe and effective vaccine against RSV have failed. A formalin-inactivated virus vaccine tested approximately 20 years ago failed to prevent infection, and actually increased the severity of disease upon subsequent RSV infection (Kim et al., 1969). Temperature-sensitive mutants have also failed as vaccines because of either insufficient attenuation or loss of immunogenicity (Kim et al., 1971; Wright et al., 1982).

Work using purified RSV proteins from RSV-infected cells (Walsh et al., 1987), or vaccination with vaccinia virus recombinants containing the F (fusion) or G (attachment) glycoprotein in mice (Stott et al., 1986; Wertz et al., 1987) or rats (Olmsted et al., 1986; Elango et al., 1986) resulted in the induction of neutralizing antibody and in a significant reduction in the replication of RSV in lung tissue. Our previous work (Wathen et al., 1989a) demonstrated that vaccination with a secreted form of the F glycoprotein (Ft), expressed in insect cells using a baculovirus vector also resulted in induction of neutralizing antibody, and in significant reduction in the titre of RSV recovered from cotton rats. The Ft glycoprotein contained the majority of the extracellular region of F (amino acids 1 to 489), but was missing the anchor region of the glycoprotein.

In the preceding paper (Wathen et al., 1989b), we described a novel chimeric glycoprotein of RSV termed FG, which was expressed in insect cells using a baculovirus vector. This glycoprotein contained the signal and extracellular regions of the F glycoprotein linked to the extracellular region of the G glycoprotein. We now report that vaccination of cotton rats with the chimeric FG glycoprotein resulted in the induction of high titres of neutralizing antibody and in protection from challenge with either serogroup A or B strains of RSV. In addition, we describe the effect of route and frequency of vaccination, and the use of adjuvants on the efficacy of FG.

METHODS

Cells and viruses. Human RSV (Long or 18537 strains) was grown in HEp-2 cell monolayers with Eagle's minimal essential medium containing 10% foetal bovine serum and 50 μg/ml of gentamicin sulphate.

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Animals. Male and female cotton rats (Sigmodon hispidus) aged 4 to 10 weeks originally obtained from the Veterinary Research Branch, Division of Research Services, National Institutes of Health were supplied by Upjohn animal rearing. The animals were used in accordance with the Guide for the Care and Use of Laboratory Animals, DHEW Publication (NIH) 85-23 and the Animal Welfare Act (U.S.A.) and subsequent amendments.

Glycoprotein preparations. Crude FG, Ft and gp50T glycoproteins were prepared from baculovirus-infected insect cells (baculo-FG, -Ft, -gp50T; Wathen et al., 1989a). The gp50T glycoprotein of pseudorabies virus (Petrovskis et al., 1986) served as the unrelated control. Briefly, spinner culture supernatant taken from baculo-FG-, baculo-Ft- or baculo-gp50T-infected cultures was centrifuged under low-speed conditions to remove insect cells and then at 100,000 g to remove the baculovirus as previously described for baculo-Ft (Wathen et al., 1989a). Clarified supernatant was then applied onto a concanavalin A-Sepharose column (Pharmacia), washed with phosphate-buffered saline (PBS) containing 0.5 M-NaCl and eluted with PBS containing 0.5 M-NaCl and 0.5 M-O-methyl mannospyranoside as previously described for the Ft glycoprotein (Wathen et al., 1989a). The fractions were assayed for either FG or Ft glycoprotein using ELISA as described (Wathen et al., 1989b) and active fractions were pooled. Protein determinations were carried out with a Bio-Rad protein assay kit using a bovine gamma globulin standard.

For semi-purified preparations of FG, cell culture supernatants were harvested as above and, following the removal of baculovirus by filtration, were applied onto a Mono S Fast Flow (Pharmacia) cation-exchange column. The column was washed with 0.05 M-NaCl in 0.02 M-MOPS and then eluted with 1.0 M-NaCl in 0.02 M-MOPS. Fractions were assayed for ELISA activity and pooled. This material was then further purified by immunoaffinity chromatography using a monoclonal antibody specific for the G glycoprotein of RSV. Briefly, ascites fluid was purified using Protein A high performance affinity chromatography. Purified monoclonal antibodies were immobilized on either diol-bonded Nucleosil 1000-10 (Alltech) or aldehyde-bonded Daltosil 1200 (Serva Biochemistries) by the Schiff base and 1,1'-carbonyldiimidazole coupling methods as previously described (Waiters, 1985), and packed into columns by the vacuum slurry method (Moore & Waiters, 1984). Pooled fractions taken from the cation exchange column were applied to the monoclonal antibody column in 0.1 M-KH₂PO₄ pH 7.0. The column was washed sequentially with 15 ml of 0.1 M-KH₂PO₄ pH 7.0 containing 1.0% Triton X-100 and 0.1 M-KCl. The FG glycoprotein was eluted from the column with either 0.2 M-lactic acid (pH 2.8) or 0.1 M-glycine–HCl pH 2.5 and immediately neutralized with Tris-HCl. Crude Ft glycoprotein which had been eluted from a concanavalin A column (described in previous paragraph) was further purified by immunoaffinity chromatography as described above, using a monoclonal antibody specific for the RSV F glycoprotein. Purity of the glycoprotein preparations was determined by densitometer scans of silver-stained SDS–polyacrylamide gels or by reverse phase chromatography.

Reverse phase chromatography. Protein samples containing the FG glycoprotein were analysed by reverse-phase high-performance liquid chromatography using a Bio-Gel TSK Phenyl RP column (Bio-Rad). Immunoaffinity-purified samples were diluted with an equal volume of water and a 100 µl volume (approximately 7 µg of protein) was injected. A 20 min linear gradient from 0.05% trifluoroacetic acid (TFA) in water to 0.05% TFA in 50:50 2-propanol : water was used to elute the protein. The absorbance of the effluent was monitored at 217 nm. The fractions containing the FG glycoprotein were detected by ELISA. The amount of FG glycoprotein in the samples was based on the percentage of the FG peak in the reverse phase profile coupled with protein determination and ELISA reactivity with monoclonal antibodies specific for the G or F protein of RSV.

Preparation of purified F and G glycoprotein from RSV-infected cells. HEp-2 cells were infected with RSV for 40 h and then washed in cold PBS, scraped and pelleted. Cellular material was detergent-lysed and affinity-purified as previously described (Walsh et al., 1987) using monoclonal antibody columns prepared as described above. For ELISA, 100 ng of purified protein was adsorbed to each well of a microtitre plate overnight at 4 °C.

Vaccinations. Crude preparations of glycoproteins were administered subcutaneously (s.c.) as a mixture with Freund's adjuvant. For the first immunization, animals received a mixture of glycoprotein in complete Freund's adjuvant. The final dosage which was also administered in incomplete adjuvant was given after another 2 weeks. For studies utilizing semi-purified glycoprotein, animals received either an intramuscular (i.m.) or intranasal (i.n.) dosage of glycoprotein administered as an alum precipitate. This material was prepared by adsorption of glycoprotein to 3% aluminium hydroxide (alhydrogel obtained from Superfos). The actual dosage of alum was 1.75 mg per animal for i.n. vaccinations and 3-5 mg per animal for i.m. vaccinations. Two weeks after the primary dosage, animals received a second dosage of alum-precipitated glycoprotein unless indicated otherwise. The volume of alum-precipitated glycoprotein administered was 0.15 to 0.3 ml for i.m. dosing and 0.1 ml for i.n. dosing. In certain experiments, a group of animals received live RSV i.n. (10⁵ p.f.u.) to serve as a positive immunizing dose. Typically, for each experiment, a group of animals received the appropriate adjuvant (alum or Freund's) to serve as the negative control.

Seroanalysis. In each animal experiment, blood was obtained from animals via retro-orbital bleeding at 10 days following the final immunization and analysed by ELISA. The assay for detection of cotton rat serum antibody
Efficacy of an RSV chimeric FG glycoprotein

RSV challenge and lung tissue harvest. In all experiments animals were challenged with 2 × 10⁶ (Long) or 7 × 10⁵ (18537) p.f.u. of RSV i.n. at 2 weeks following the final immunization as described (Wathen et al., 1989a). Four days after challenge, lung tissues were removed and processed as 10% homogenates (w/v) in cell culture medium. Virus titre was determined by plaque assay on HEp-2 cells and was expressed as the average p.f.u./g of lung tissue.

Statistical analysis. For each experiment, treatment groups were compared with their appropriate negative controls for statistical differences in the titre of RSV found in the lungs of infected animals. The student's t-test analysis was used and P values are shown.

RESULTS

Cotton rats were vaccinated over a period of 7 weeks with crude glycoprotein material prepared with Freund's adjuvant (see Table 1). Serum antibody responses were measured by ELISA, which detected binding to RSV-infected target cells, and by neutralization of the virus. Animals which had been vaccinated with the chimeric FG glycoprotein or Ft had nearly equivalent ELISA titres. Animals vaccinated with the FG glycoprotein had fivefold higher neutralizing titres. Two weeks after the third vaccination, animals were challenged with RSV (Long). FG-vaccinated animals had a lower incidence of infection and a lower level of virus was recovered from the lungs of infected animals as compared to those animals vaccinated with Ft, although the difference was not statistically significant. Both FG- and Ft-vaccinated animals had significantly reduced levels of virus in their lungs as compared to the animals which received the unrelated glycoprotein gp50T. Animals vaccinated with gp50T had 100% incidence of infection and over 10⁴ p.f.u./g of RSV was recovered from lung tissue.

A similar experiment was performed to measure the effect of vaccination with FG or Ft against RSV serogroup B (18537). In this study, animals were vaccinated twice with crude glycoprotein material in the presence of Freund's adjuvant (see Table 1). FG-vaccinated animals had higher neutralizing antibody responses to RSV (18537), than did those animals vaccinated with Ft. However, the incidence of infection with RSV was similar for the FG- and Ft-vaccinated animals. Both groups of animals were significantly protected from RSV (18537) as compared to the gp50T controls which had 100% incidence and over 10⁴ p.f.u./g of virus in their lungs. The FG-vaccinated animals had lower levels of RSV than did those vaccinated with Ft but this difference was not statistically significant.

To provide a more quantitative comparison, the FG and Ft glycoproteins were further purified by immunoaffinity chromatography. The purity of the Ft glycoprotein was determined by integration of peaks from densitometer scans of silver-stained SDS-polyacrylamide gels (data not shown). Because of the extensive heterogeneity, the purity of the FG glycoprotein could not be readily quantified by SDS–PAGE (Wathen et al., 1989b). FG preparations were therefore analysed by reverse phase chromatography (Fig. 1). The reverse phase chromatographic peak containing the FG glycoprotein was determined by ELISA and SDS–PAGE. The purity of the FG preparation was determined by comparing the area under the FG reverse phase peak to the remaining chromatogram. The FG preparation illustrated in Fig. 1 was approximately 50% pure.

Immunoaffinity-purified material was taken and used in animal vaccination studies. Semi-purified FG or Ft glycoprotein was administered twice to animals along with either Freund's adjuvant or with alum in the form of a precipitate. In this series of experiments (Table 2), the animals receiving alum-precipitated saline served as negative controls and animals receiving 2 × 10⁴ p.f.u. live RSV (Long) administered i.n. served as the positive controls. Different preparations of FG and Ft were used. The purity of the FG preparations ranged from 2-5% to 50%. The Ft preparation was approximately 33% pure. Table 2 indicates the total protein per dose as well as the estimated amount of specific glycoprotein to allow for comparison between the groups. Vaccination with FG prepared as an alum precipitate or with Freund's adjuvant at
Fig. 1. Reverse phase chromatograph of the FG glycoprotein. Protein samples were analysed on a Bio-Gel TSK Phenyl RP column using a linear gradient from 0.05% TFA to 0.05% TFA in 50:50 2-propanol: water. Proteins were detected by absorbance at 217 nm. A chromatogram where only solvent was run was included to show background absorbance and solvent impurities. Corresponding ELISA activities to detect the FG glycoprotein in individual fractions using a pool of monoclonal antibodies specific for the F and G portions of FG were determined. ELISA values for fractions 1 to 5 are 0.018, 0.012, 0.007, 1.303 and 0.012, respectively.

Table 1. Immune response and protection of cotton rats vaccinated with crude FG or Ft glycoprotein material

<table>
<thead>
<tr>
<th>Challenge virus*</th>
<th>Antigen†</th>
<th>ELISA titre‡</th>
<th>Neutralizing titre§</th>
<th>No. infected/total no. animals¶</th>
<th>Average lung titre (log10)†¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long FG</td>
<td>FG</td>
<td>12950</td>
<td>1970</td>
<td>1/18 (6)</td>
<td>2.2</td>
</tr>
<tr>
<td>Long Ft</td>
<td>Ft</td>
<td>12800</td>
<td>360</td>
<td>7/18 (38)</td>
<td>3.6 ± 0.23</td>
</tr>
<tr>
<td>Long gp50T</td>
<td>gp50T</td>
<td>&lt;100</td>
<td>&lt;20</td>
<td>16/16 (100)</td>
<td>5.1 ± 0.21</td>
</tr>
<tr>
<td>18537 FG</td>
<td>FG</td>
<td>10900</td>
<td>910</td>
<td>4/18 (22)</td>
<td>2.2 ± 0.12</td>
</tr>
<tr>
<td>18537 Ft</td>
<td>Ft</td>
<td>11900</td>
<td>220</td>
<td>5/16 (31)</td>
<td>3.0 ± 0.23</td>
</tr>
<tr>
<td>18537 gp50T</td>
<td>gp50T</td>
<td>&lt;100</td>
<td>&lt;20</td>
<td>18/18 (100)</td>
<td>4.2 ± 0.24</td>
</tr>
</tbody>
</table>

* Animals were challenged 2 weeks following the final vaccination with 2 × 10⁶ p.f.u. of a serogroup A virus (Long) or 7 × 10⁵ p.f.u. of a serogroup B virus (18537).
† Crude glycoprotein material estimated to be <1% pure. Animals received two or three vaccinations. Data from two experiments.
‡ ELISA titre (average of 10 to 12 animals) expressed as the reciprocal of the serum dilution giving 50% maximum binding against RSV (Long)-infected target cells. Animals were bled 10 days after the final vaccination.
§ Neutralizing titre (average of 10 to 12 animals) expressed as the reciprocal of the dilution that reduced viral c.p.e. by 50% relative to unneutralized controls against homologous virus.
¶ Number of rats with detectable virus in their lungs at removal. The limit of detectability was 10¹⁷ p.f.u./g of lung.
† Standard error of the mean is indicated for groups with more than one infected animal. P value comparisons of lung titres of infected animals for FG- and Ft-vaccinated groups compared to gp50T control animals were <0.004 for both serogroup A or B RSV. Comparisons of FG and Ft were not statistically significant (P value <0.29 for Long strain and <0.14 for 18537 strain).

Dosage levels as low as 0.05 μg of FG were effective in induction of both serum ELISA-reactive and neutralizing antibody, and resulted in significant reduction in the titre of RSV found in lung tissue. In addition, as compared to the animals which were vaccinated i.n. with live RSV (positive controls), FG-vaccinated animals had much higher levels of neutralizing antibody. The amount of extraneous protein in the preparation did not affect the potency of the FG glycoprotein. Preparations with different levels of purity were equally effective at inducing antibodies and protecting against RSV challenge when the dosages were equalized for the amount of FG glycoprotein. The Ft glycoprotein was not as effective an immunogen as FG. Vaccination with 2.5 μg Ft glycoprotein prepared as an alum precipitate induced lower levels of neutralizing antibody compared to lower doses of FG, but did significantly reduce the titre of RSV in lung tissue by over 100-fold. The incidence of infection with RSV was also lower for the
Table 2. Vaccination with semi-purified FG or Ft glycoprotein using alum as an adjuvant

<table>
<thead>
<tr>
<th>Antigen*</th>
<th>Protein (μg)</th>
<th>Glycoprotein (μg)†</th>
<th>ELISA titre‡</th>
<th>Neutralizing titre§</th>
<th>No. infected/total no. animals</th>
<th>Average lung titre (log10)¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>FG-CFA</td>
<td>40</td>
<td>1.0</td>
<td>12300</td>
<td>5029</td>
<td>1/7</td>
<td>2.0</td>
</tr>
<tr>
<td>FG-Alum</td>
<td>2</td>
<td>0.05</td>
<td>6500</td>
<td>2560</td>
<td>3/7</td>
<td>24 ± 0.17</td>
</tr>
<tr>
<td>FG-Alum</td>
<td>8</td>
<td>0.20</td>
<td>18100</td>
<td>2027</td>
<td>0/6</td>
<td>-</td>
</tr>
<tr>
<td>FG-Alum</td>
<td>40</td>
<td>1.0</td>
<td>18200</td>
<td>2834</td>
<td>0/7</td>
<td>-</td>
</tr>
<tr>
<td>FG-Alum</td>
<td>0.4</td>
<td>0.2</td>
<td>11800</td>
<td>1344</td>
<td>0/7</td>
<td>-</td>
</tr>
<tr>
<td>FG-Alum</td>
<td>2.0</td>
<td>1.0</td>
<td>32100</td>
<td>5620</td>
<td>0/7</td>
<td>-</td>
</tr>
<tr>
<td>Ft-Alum</td>
<td>8</td>
<td>2.5</td>
<td>8200</td>
<td>194</td>
<td>4/7</td>
<td>2.5 ± 0.34</td>
</tr>
<tr>
<td>Ft-Alum</td>
<td>40</td>
<td>12</td>
<td>11150</td>
<td>263</td>
<td>2/7</td>
<td>2.3 ± 0.24</td>
</tr>
<tr>
<td>RSV (104 p.f.u.)</td>
<td>4250</td>
<td></td>
<td>217</td>
<td>1/7</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>Alum</td>
<td>&lt;400</td>
<td>&lt;20</td>
<td>14/14</td>
<td>5.0</td>
<td>0 ± 0.61</td>
<td></td>
</tr>
</tbody>
</table>

* Alum-containing material was administered i.m. FG-CFA was administered s.c. and RSV (Long) was administered i.n. All animals were challenged with RSV (Long) following two vaccinations of glycoprotein. Data from two experiments.
† The FG glycoprotein preparation was 2-5 or 50% pure. Ft was 33% pure.
‡ ELISA titre (average of five to six animals) expressed as the reciprocal of the serum dilution giving 50% maximum binding against RSV (Long) target cells. Animals were bled 10 days following the final vaccination.
§ Neutralizing titre (average of five to six animals) expressed as the reciprocal of the dilution that reduced viral c.p.e. by 50% relative to unneutralized controls (alum) against RSV (Long).
¶ Number of rats with detectable virus in their lungs at removal. The limit of detectability was 101.7 p.f.u./g of lung.

Table 3. Serological response of immune rat sera to RSV F or G glycoprotein

<table>
<thead>
<tr>
<th>Group*</th>
<th>ELISA titre endpoint†</th>
<th>Anti-F</th>
<th>Anti-G</th>
<th>Ratio (F : G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FG</td>
<td>28000</td>
<td>1300</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Ft</td>
<td>32000</td>
<td>250</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>RSV (104 p.f.u.)</td>
<td>4700</td>
<td>380</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>gp50T</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>NA†</td>
<td></td>
</tr>
</tbody>
</table>

* Sera taken from vaccinated animals and reacted against 100 ng of purified F or G glycoprotein by ELISA.
† ELISA titre (average of three to five animals) expressed as the reciprocal of the serum dilution giving five times that found for PBS against the same antigen.
‡ NA, Not applicable.

animals vaccinated with FG than for those vaccinated with Ft. On a μg per dose basis, FG appeared to have approximately 50 times the immunizing activity of Ft in terms of comparable levels of antibody and protection of the lung from RSV challenge.

Sera taken from FG- or Ft-vaccinated animals were analysed for binding to F or G glycoproteins purified by immunoaffinity chromatography from RSV-infected cells. Table 3 summarizes the ELISA binding titres of FG- or Ft-vaccinated animals indicating that both groups of animals had high titres of serum antibody against the F glycoprotein. In addition, sera taken from animals vaccinated with live RSV recognized the F glycoprotein. Sera taken from Ft-vaccinated animals bound to the G glycoprotein with a titre slightly above background, probably because of low level contamination of the G antigen with F glycoprotein. Sera taken from FG- or RSV-vaccinated animals bound to the G glycoprotein at levels significantly above background. However, the binding reaction of sera from FG- and RSV-vaccinated animals to the G glycoprotein was well below that to F. The ratio of ELISA titres to the F and G antigens was similar for the FG and RSV vaccinees. Sera taken from animals vaccinated with the unrelated glycoprotein gp50T did not respond to either RSV F or G glycoproteins.
Our efforts focused on testing the FG glycoprotein in animals as a potential vaccine against RSV. The effect of single compared to multiple vaccinations was determined (Table 4). High titres of serum ELISA antibody directed toward RSV-infected target cells were found following one vaccination with 0.05, 0.2 or 1.0 µg of FG. These levels were found to increase following the second vaccination, reflecting development of a strong secondary response. Development of high titres of neutralizing antibody were dependent upon the animals receiving two vaccinations of FG. A single vaccination with as little as 0.05 µg of FG resulted in significant reduction of RSV in lung tissue. Two vaccinations with as low as 0.05 µg of FG protected 100% of the animals.

In early experiments (Table 2), we had shown that vaccination with FG was effective against RSV when prepared either with Freund’s adjuvant and administered s.c. or when prepared as an
Efficacy of an RSV chimeric FG glycoprotein

alum precipitate and administered i.m. To extend these results, we examined the effect of vaccination with alum-precipitated, semi-purified FG via the i.n. route and compared it to that of i.m. vaccination (Table 5). Animals vaccinated i.n. with FG had serum ELISA antibody levels comparable to those vaccinated i.m. The level of neutralizing antibody induced following i.n. vaccination with FG was below that found following i.m. or s.c. vaccination. Despite this, animals which had received two i.n. vaccinations with FG had significantly less virus in their lungs (nearly 1000-fold) compared to the alum negative controls.

DISCUSSION

We have compared the efficacy of FG, a novel chimeric glycoprotein which contains the immunogenic regions of the F and G glycoproteins of RSV, to that of Ft, a secreted form of the F glycoprotein (Wathen et al., 1989a) using an experimental model of RSV in cotton rats. This report demonstrated that vaccination with FG was superior to vaccination of animals with Ft for the following reasons: (i) higher neutralizing antibody titres were found in FG vaccinees, (ii) the incidence of infection with RSV was lower for FG vaccinees, (iii) the titre of virus recovered from the lungs of infected animals was lower for the FG-vaccinated group and (iv) on a μg basis FG was in the order of 50 times more potent than Ft.

Vaccination of cotton rats with either the FG or Ft glycoprotein significantly reduced the titre of RSV recovered from animals which were challenged with either A or B serotype virus strains of RSV. FG-vaccinated animals when challenged with serogroup A appeared to have a lower incidence of infection compared to Ft-vaccinated animals against the same virus. However, both FG- and Ft-vaccinated animals had a similar incidence of infection in response to challenge with serogroup B virus. Since the G glycoprotein demonstrates substantial serogroup divergence while the F glycoproteins of the two serogroups are very closely related immunologically (Walsh et al., 1987), the above data suggest that the G portion of the chimeric molecule (originally derived from a serogroup A virus cDNA) is contributing to immunity when the animals are challenged with serogroup A virus. However, larger groups of animals would be needed to verify these observations statistically. In addition, animals which were vaccinated with crude FG or Ft had high titres of serum antibodies directed against the F glycoprotein of RSV purified from infected cells, whereas only FG-vaccinated animals had a measurable response to the G glycoprotein of RSV. The response to the latter protein in FG-vaccinated animals was substantially lower than the response to F. However, the response to G was similarly low compared to F in animals vaccinated i.n. with live RSV. The ratios of binding to F compared to binding to G for animals vaccinated with live RSV and animals vaccinated with FG were similar. The lower antibody titre to the G antigen may reflect a lower immunological response to the G glycoprotein following vaccination. Others (Walsh et al., 1987) have shown that the serological response of animals to G was much lower than to F following immunization. Alternatively, the lower binding response of antisera to G may reflect partial denaturation of the RSV G glycoprotein during immunoaffinity purification, or poor adsorption of the G glycoprotein to the microtitre plate used for the ELISA.

Studies using semi-purified (2-5% pure) FG glycoprotein (Table 2), showed that a single vaccination with 8 μg of protein (0.2 μg of FG) or two vaccinations with 2 μg (0.05 μg of FG) were sufficient to protect 85 to 100% of the animals. As little as 0.4 μg of a preparation of FG which was estimated to be 50% pure (Table 2) administered with alum protected 100% of the animals from RSV challenge. Vaccination with FG was effective in protection of cotton rats when administered s.c. with Freund's adjuvant, or when administered by the i.n. or i.m. route with alum (Tables 2 and 5).

In animals vaccinated with FG, the levels of serum (ELISA) antibody directed against RSV-infected target cells and neutralizing antibody directed against the virus were generally correlated inversely with both the incidence and titre of RSV found in lung tissue. However, this was not the case in animals vaccinated with a single dose of FG. At higher dose levels, the lungs of these animals were completely protected from viral challenge despite having low neutralizing antibody titres. Animals which had been vaccinated twice with FG had higher levels of ELISA and neutralizing antibody than those receiving only one dose, reflecting the development of a
secondary immune response. The ability to protect animals in the presence of low levels of neutralizing antibodies indicates that other factors are also important for protection in the cotton rat model. In other studies in cotton rats (R. J. Brideau & M. W. Wathen, unpublished results), we have found that when lymphocytes taken from animals immunized with semi-purified FG glycoprotein were exposed to sucrose gradient-purified RSV in vitro, they responded in an antigen-driven manner as measured by proliferation and production of interleukin 2, reflecting the induction of T cell responsiveness. Taken together, these results suggest that vaccination of animals with FG results in induction of both cellular and antibody-mediated responsiveness which allow for protection against experimental RSV challenge.

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REFERENCES


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