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Immunosuppression of the Antibody Response to Respiratory Syncytial Virus (RSV) by Pre-existing Serum Antibodies: Partial Prevention by Topical Infection of the Respiratory Tract with Vaccinia Virus–RSV Recombinants

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SUMMARY

Immunization strategies to prevent respiratory syncytial virus (RSV) disease will involve immunization of infants less than 2 months of age who possess maternally derived RSV antibodies. Vaccinia–RSV recombinant viruses are useful tools for defining parameters important in immunization against RSV and also are being considered as live virus vaccines for use in humans. Previous studies demonstrated that passively acquired RSV antibodies can suppress the immune response and the protective efficacy of vaccinia–RSV recombinants administered by the intradermal route. The present study demonstrates that the suppressive effects of passively acquired antibody on immunity induced by intradermally administered vaccinia–RSV recombinants in cotton rats can be partially overcome by administration of the recombinants by the intranasal route.

Respiratory syncytial virus (RSV) is unique among respiratory viruses in causing its highest incidence of serious lower respiratory tract disease at 2 months of age (McIntosh & Chanock, 1985; Kim et al., 1973). For this reason, strategies to prevent RSV disease will involve immunization of very young infants. Since RSV is a ubiquitous viral pathogen and reinfection with RSV is common, human adults typically have high levels of antibody to the RSV glycoprotein antigens, and young infants thus possess high levels of maternally derived RSV-specific serum antibodies. RSV encodes two glycoproteins, the attachment glycoprotein G and the fusion glycoprotein F, which are the major virus protective antigens (Olmsted et al., 1986; Wertz et al., 1987; Walsh et al., 1984). Recent evidence suggests that the presence of maternally derived antibodies at the time of RSV infection can decrease the antibody response of human infants or bovine calves to the two RSV glycoproteins (Murphy et al., 1986a, b; Kimman et al., 1987).

This phenomenon of antibody-mediated immunosuppression has recently been studied in cotton rats (Sigmodon hispidus) in which hyperimmune RSV antiserum passively transferred prior to intradermal infection with vaccinia–RSV glycoprotein recombinant viruses suppressed the antibody response to RSV glycoproteins but not to vaccinia virus antigens (Murphy et al., 1988). Similar observations were made using vaccinia–influenza virus haemagglutinin recombinants (Johnson et al., 1988). The cotton rats which had their immune response suppressed by passively transferred RSV antibodies were more susceptible to infection with RSV than were animals inoculated with control serum lacking RSV antibodies (Murphy et al., 1988). Furthermore, many of the immunosuppressed animals infected with the vaccinia–RSV recombinant viruses developed antibodies to the RSV glycoproteins which had abnormally low neutralizing activity.
Since subunit paramyxovirus vaccines are being considered for human use, administered either in the form of a live recombinant virus that expresses protective viral glycoproteins or as purified viral glycoproteins, it would be of interest to evaluate their ability to stimulate an immune response in the presence of passively transferred antibodies (Walsh et al., 1987; Ray & Compans, 1987; Olmsted et al., 1988; Wertz et al., 1987; Spriggs et al., 1987; Stott et al., 1986).

In the present study, we examined the effect of passively derived serum RSV antibodies on the antibody response to vaccinia–RSV recombinant viruses administered intranasally. The experimental findings indicated that the suppressive effects of passively acquired serum RSV antibodies on the immune response to intradermally administered live vaccinia–RSV recombinant viruses could be largely overcome by administering the vaccinia–RSV recombinants intranasally. Furthermore, the animals that passively received RSV antibodies prior to intranasal infection with the vaccinia–RSV recombinants exhibited a high level of resistance to RSV infection in both the upper and lower respiratory tracts.

The design of this experiment involved (i) passive transfer on day 1 of 4 ml of RSV hyperimmune cotton rat antiserum or normal cotton rat serum by intraperitoneal inoculation of 3- to 4-week-old cotton rats (approx. 40 g), (ii) administration on day 0 of 10^6 p.f.u. each of Vac-F (vaccinia virus recombinant expressing the RSV F glycoprotein) and Vac-G (vaccinia virus recombinant expressing the RSV G glycoprotein) intranasally in a total inoculum of 0.1 ml (Olmsted et al., 1986, 1988), (iii) collection of blood from animals on days 0 and 55 for quantification of RSV glycoprotein-binding antibodies by F- or G-specific ELISA (Murphy et al., 1986), RSV-neutralizing antibodies by plaque reduction assay (Prince et al., 1986), and vaccinia virus-specific antibodies by ELISA (Murphy et al., 1988), (iv) challenge of animals intranasally on day 56 with 10^5 p.f.u. of RSV strain A2 (0.1 ml) and (v) harvest of lungs and nasal turbinates 4 days later for quantification of virus replication. The methods for preparation and titration of the vaccinia–RSV F and G recombinants and for determining the titre of RSV in lungs and nasal turbinates have been described (Prince et al., 1986). The cotton rat hyperimmune antiserum was derived from cotton rats that had been infected intranasally and rechallenged twice with 10^5 TCID_{50} of RSV strain A2. This antiserum had a 60% plaque reduction titre of approximately 1 : 2000. Since the half-life of passively transferred cotton rat neutralizing antibodies is 6 days, the titre of passively transferred RSV antibodies diminished to almost background levels on day 55 allowing the antibody responses to the vaccinia virus-expressed F and G glycoproteins to be detected unambiguously (Murphy et al., 1988).

Animals that received undiluted RSV immune serum (group B, Table 1) possessed titres of RSV antibodies in the preinfection serum comparable to levels of maternally derived RSV antibodies present in human infants 1 to 2 months of age (Murphy et al., 1986). With regard to RSV glycoprotein-binding antibodies, the frequency and magnitude of the serum RSV F and G antibody responses in these animals following administration of Vac-F and Vac-G recombinants intranasally (group B) was only slightly less than that of the group of animals that received undiluted control serum (group A). The differences in the F and G antibody responses between these groups was significant only for the magnitude of the G glycoprotein response. However, with regard to RSV-neutralizing antibodies, responses were significantly decreased in animals (groups B and C) that received undiluted RSV serum or RSV serum diluted 1 : 3. This indicated that there was a dissociation between the levels of binding and neutralizing antibodies, consistent with observations in previous studies (Murphy et al., 1986, 1988). The immune responses of Vac-F- and Vac-G-infected animals that received a dilution of RSV immune sera of 1 : 9 to 1 : 81 (groups D to F) were similar in each respect to that of control animals (group A), showing that an immunosuppressive effect on the intranasal immunization with Vac-F and Vac-G was observed only with high levels of passive serum RSV antibodies.

Animals that had been infected by vaccinia–RSV recombinant viruses intranasally were resistant to RSV challenge in both the upper and lower respiratory tract regardless of the amount of RSV antibodies administered on day 0 (Table 2). Resistance to RSV challenge was only slightly decreased in the nasal turbinates of animals that had received the undiluted RSV antiserum (group B, Table 2), but in the remaining groups (C to F) resistance was equivalent to that of non-immunosuppressed immunized animals (group A).
Table 1. Effect of passive transfer of RSV immune serum on the antibody response of cotton rats infected intranasally with vaccinia–RSV G and F recombinant viruses

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum passive transfer*</th>
<th>Vaccinia virus administered (i.n.)†</th>
<th>No. of animals</th>
<th>Serum ELISA antibody response to indicated antigen on day 0 or 55</th>
<th>Serum neutralizing antibody response to RSV on day 0 or 55</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vaccinia titre (reciprocal mean log₂ ± S.E.)</td>
<td>Percentage</td>
<td>Vaccinia titre (reciprocal mean log₂ ± S.E.)</td>
<td>Percentage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>55</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>A</td>
<td>Control</td>
<td>Vac-G + Vac-F</td>
<td>6</td>
<td>6.0 ± 0.4 15.2 ± 0.3 100</td>
<td>6.6 ± 0.4 14.9 ± 0.3 100</td>
</tr>
<tr>
<td>B</td>
<td>RSV undiluted</td>
<td>Vac-G + Vac-F</td>
<td>8</td>
<td>16.0 ± 0.4 14.7 ± 0.8 83</td>
<td>16.4 ± 0.4 12.5 ± 0.8 § 63</td>
</tr>
<tr>
<td>C</td>
<td>RSV 1:3</td>
<td>Vac-G + Vac-F</td>
<td>8</td>
<td>15.2 ± 0.4 17.2 ± 0.4 100</td>
<td>15.7 ± 0.8 15.0 ± 0.8 100</td>
</tr>
<tr>
<td>D</td>
<td>RSV 1:9</td>
<td>Vac-G + Vac-F</td>
<td>5</td>
<td>13.2 ± 0.6 17.2 ± 0 100</td>
<td>11.7 ± 0.4 15.2 ± 0.6 100</td>
</tr>
<tr>
<td>E</td>
<td>RSV 1:27</td>
<td>Vac-G + Vac-F</td>
<td>2</td>
<td>9.3 ± 0.7 17.2 ± 0 100</td>
<td>11.5 ± 0 15.2 ± 0 100</td>
</tr>
<tr>
<td>F</td>
<td>RSV 1:81</td>
<td>Vac-G + Vac-F</td>
<td>3</td>
<td>10.6 ± 0.7 16.5 ± 0.7 100</td>
<td>9.3 ± 0 14.6 ± 0 100</td>
</tr>
<tr>
<td>G</td>
<td>Control</td>
<td>Vac-VSC-14</td>
<td>5</td>
<td>8.1 ± 0.5 10.5 ± 1.5</td>
<td>NA§</td>
</tr>
</tbody>
</table>

* Four ml of RSV immune or control serum was administered intraperitoneally on day −1.
† Vaccinia–RSV recombinant virus expressing the F (Vac-F) or G (Vac-G) glycoprotein or a recombinant vaccinia virus expressing an unrelated antigen (Vac-VSC-14) were administered (10⁵ p.f.u. each in a 0.1 ml inoculum) intranasally on day 0.
‡ An animal was considered to have a response if its antibody titre on day 55 was fourfold or higher than that of control animals (group G). S.E., Standard error.
§ Statistically significant difference (Student’s t-test), P < 0.01.
After Vac-VSC-14 infection there is a slight increase in antibody titre to the F and G glycoproteins for reasons that are undefined.
¶ Not applicable.
** Statistically significant difference (Student’s t-test) between group A and B or C, P < 0.001.
It was previously observed that passively transferred RSV antibodies suppressed the frequency and magnitude of antibody response to RSV glycoproteins induced by the intradermal administration of Vac-F and Vac-G RSV recombinant viruses. These previous results for Vac-F administered intradermally are compared to those in the present study (Table 3). In comparison to control animals, the frequency of response to the F glycoprotein was decreased 67% in animals that were intradermally infected with Vac-F and Vac-G after receiving undiluted RSV antisem. Similarly, the magnitude of the ELISA F antibody response was reduced 23-fold. In the present study in which the same RSV immune serum was used, the frequency of the F antibody response of passively immunized animals to Vac-F and Vac-G given intranasally was decreased only 17% in comparison to animals receiving control serum. Furthermore, the magnitude of the immune response was decreased only 1.4-fold. Similar results were found for the G antibody response to the RSV G glycoprotein. However, the passive antibody selectively suppressed the neutralizing antibody response to Vac-F and Vac-G given intranasally as had been observed previously for animals infected intradermally with Vac-F and Vac-G recombinant virus (Murphy et al., 1988).

The immunosuppression mediated by the passive RSV antibodies observed previously in animals infected intradermally with Vac-F and Vac-G was associated with a failure to develop resistance to challenge with RSV, whereas complete resistance to pulmonary virus replication was seen in passively immunized animals infected with Vac-F and Vac-G intranasally. It is likely that two factors contributed to the greater degree of resistance in the passively immunized, intranasally infected animals. First, because the level of immunosuppression was greatly reduced in the intranasally compared to intradermally infected animals, more RSV antibodies were available at the time of challenge to restrict replication of virus. This suggests that the response of immunoglobin-producing cells in the respiratory tract was less readily suppressed by passive serum RSV antibodies than were corresponding lymphocytes outside the respiratory tract. Second, it is likely that local respiratory tract immunity, involving RSV-specific IgA antibody as well as possible cellular factors (Bangham et al., 1986; Pemberton et al., 1987) were more efficiently induced by the replication of vaccinia recombinant virus in the respiratory tract of the intranasally infected cotton rats. Consistent with this latter suggestion is the observation of a 90-fold reduction in virus replication in the nasal turbinates of passively immunized (1:3 dilution of serum) animals that were infected intranasally with Vac-F and Vac-G compared to an only twofold reduction in similarly immunized animals infected intradermally.
Table 3. Antibody response to RSV F glycoprotein is less subject to suppression by pre-existing RSV antibodies during nasal infection by a vaccinia–RSV F recombinant than during intradermal infection

<table>
<thead>
<tr>
<th>Cotton rat serum inoculated 1 day before infection by vaccinia–RSV</th>
<th>Percentage of animals which developed an F antibody response (ELISA) 55 days after vaccinia–RSV inoculation</th>
<th>Fold reduction in F antibody titre (ELISA) of animals with pre-existing RSV antibodies compared to controls*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Undiluted</td>
<td>100 100</td>
<td>–</td>
</tr>
<tr>
<td>RSV immune Undiluted</td>
<td>100 100</td>
<td>–</td>
</tr>
<tr>
<td>1:3</td>
<td>33 83</td>
<td>23:0 1:4</td>
</tr>
<tr>
<td>1:9</td>
<td>57 100</td>
<td>9:2 0</td>
</tr>
<tr>
<td>1:27</td>
<td>82 100</td>
<td>6:5 0</td>
</tr>
<tr>
<td>1:81</td>
<td>100 100</td>
<td>2:7 0</td>
</tr>
</tbody>
</table>

* Response to vaccinia virus was not affected by pre-existing RSV antibodies.

The implications of this work for the use of vaccinia recombinant viruses for prevention of RSV disease in neonates are clear. The decreased immunogenicity and efficacy of vaccinia–RSV recombinants administered intradermally to infants with passive serum antibodies would limit their usefulness as vaccines. It is likely that the phenomenon of immunosuppression involving the quality as well as the magnitude of the antibody response will be a factor for other candidate RSV vaccines administered parenterally. However, the high level of efficacy of these immunogens expressed during topical infection of the respiratory tract, even in animals with high levels of passive antibody, suggests that this route of immunization should be explored further as a general strategy for RSV immunoprophylaxis. Such studies should be initiated in small laboratory animals and non-human primates for safety, immunogenicity and efficacy, before studies in humans are considered (Olmsted et al., 1988).

REFERENCES


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