Protection of hamsters against experimental mumps virus (MuV) infection by antibodies raised against the MuV surface glycoproteins expressed from recombinant vaccinia virus vectors

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Mumps virus (MuV), a paramyxovirus, is the causative agent of a benign and often asymptomatic childhood disease associated with infection of parotid glands (Wolinsky & Waxham, 1990). In a number of cases, however, severe complications may occur such as deafness and meningitis. Two live vaccine strains of MuV have been used widely; one of these, however, the Urabe strain, was recently discontinued, despite its efficacy, because it caused several cases of post-vaccination meningitis (Brown et al., 1991). The second strain, Jeryl Lynn, although less efficient than the previous one, remains in use since it displays greater apparent safety (Nokes & Anderson, 1991). MuV possesses two surface glycoproteins in the viral envelope, the fusion (F) and the hemagglutinin–neuraminidase (HN) proteins. The former mediates fusion of viral and cellular membranes whereas the latter promotes viral attachment to cellular receptors. The F protein is initially synthesized as a precursor (F0) which is subsequently processed into F1 and F2 subunits, held together by a disulphide bridge. Both F and HN proteins carry membrane anchorage domains either in the C-terminal (F) or in the N-terminal part (HN) of the molecule (Elango et al., 1989; Kovamees et al., 1989; Waxham et al., 1987, 1988). In the present study, we attempted to express MuV F and HN proteins via vaccinia virus recombinants in order to evaluate their immunogenic properties in laboratory animals. This type of information is particularly relevant since the F and HN proteins constitute major targets for the development of subunit vaccines for human use.

Recombinant vaccinia viruses carrying the complete sequences coding for the F (Elango et al., 1989) and HN (Kovamees et al., 1989) proteins of the SBL-1 strain of MuV were constructed according to the procedure described by Mackett & Smith (1986). Cloned DNAs were under the control of the vaccinia virus P7.5 promoter and were introduced into the vaccinia virus (WR strain) via the transfer vector pULB5213, a derivative of pSC11 (Chakrabarti et al., 1985). Plasmids pNIV3205 (F) and pNIV3208 (HN) were used to generate vaccinia virus recombinants VV3205 and VV3208.

CVI cells infected with the recombinant vaccinia viruses were tested for the expression of the F and HN proteins. In the first assay, we used an ELISA based on anti-F or anti-HN MAbs (MAbs 5414 and 2072, respectively; Orvell, 1984) and a rabbit anti-MuV infected serum (produced in our laboratory). The results showed that the recombinant vaccinia viruses carrying engineered F and HN coding sequences produced immunoreactive proteins, which remained cell-associated.

In a second series of experiments, we performed radioimmunoprecipitation using MAbS 5414 (anti-F) and 2072 (anti-HN) on crude CVI cell extracts derived from infected cultures (m.o.i. 5) labelled either for 12 h with [3H]glucosamine or for 5 h with [35S]methionine. Immunoprecipitates were analysed on 12 or 17% SDS–polyacrylamide gels using autoradiography. As seen in
Fig. 1, F and HN polypeptides synthesized in VV3208- (HN) or VV3205- (F) infected cells, labelled with \([3H]\)glucosamine, and immunoprecipitated with anti-F or anti-HN MAbs. Immunoprecipitates were separated on 17% SDS-polyacrylamide gels and autoradiographed. Lane 1, molecular mass markers; lane 2, VV3208-infected cell lysate immunoprecipitated with anti-HN IgG (ascites 2075, Orvell, 1984); lane 3, VV3205-infected cell lysate immunoprecipitated with anti-F IgG (ascites 2159, Orvell, 1984).

Fig. 1, under reducing conditions the complete \([3H]\)glucosamine-labelled F protein was present as two subunits, F1 and F2, having the expected molecular masses 60 kDa and 20 kDa, respectively, and the complete HN protein appeared as a monomer of 75 kDa, also as expected. This experiment further indicated that both proteins were glycosylated since \([3H]\)glucosamine was incorporated.

In order to evaluate the immunogenicity of F and HN recombinants in laboratory animals, hamsters were inoculated with VV3205 or VV3208, twice at 21 day intervals (9 x 10^9 p.f.u., intradermally). The sera, Hanti F3205 and Hanti HN3208, were collected 46 days after the primary inoculation and tested by ELISA (see text). IgG titres expressed as the reciprocal of the serum dilution giving 50% maximum binding against MuV total antigens.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Animal No.</th>
<th>Antibody</th>
<th>ELISA* (Reciprocal antibody titre)</th>
<th>Neutralization† (dilution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VV3208 (HN)</td>
<td>1</td>
<td>Hanti HN 3208</td>
<td>40960</td>
<td>1:80</td>
</tr>
<tr>
<td></td>
<td>2</td>
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<td>1:40</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td>40960</td>
<td>1:80</td>
</tr>
<tr>
<td>VV3205 (F)</td>
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</tr>
<tr>
<td></td>
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<td></td>
<td>40960</td>
<td>1:640</td>
</tr>
<tr>
<td></td>
<td>6</td>
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<td>10240</td>
<td>1:320</td>
</tr>
<tr>
<td>VV TK ‡</td>
<td>7</td>
<td>Hanti βgal</td>
<td>1280</td>
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<tr>
<td></td>
<td>8</td>
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<td>1280</td>
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<td></td>
<td>9</td>
<td></td>
<td>1280</td>
<td>&lt;1:10</td>
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</tbody>
</table>

* Animals received two intradermal doses (9 x 10^9 p.f.u.) of each vaccinia virus studied. Sera were collected 46 days after the first inoculation and tested by ELISA (see text). IgG titres expressed as the reciprocal of the serum dilution giving 50% maximum binding against MuV total antigens.
† MuV serum-neutralizing antibody titres were determined by 50% plaque reduction assay.
‡ Recombinant thymidine kinase-negative vaccinia virus expressing β-galactosidase.

Table 1. MuV-specific antibody titres and neutralization capacity in hamsters immunized with VV3205 (F) and VV3208 (HN)

the use of the o-phenylenediamine (OPD) chromogenic substrate (Sigma) and their absorbance was measured at 492 nm. The results, shown in Table 1, demonstrated a specific reaction of the Hanti F 3205 and Hanti HN 3208 sera to MuV antigens whereas non-immune serum and serum derived from an animal inoculated with a thymidine kinase negative-vaccinia virus (negative control) gave only background values. Hamster sera were also tested for MuV neutralizing activity. Neutralization assays were performed as described previously (Orvell, 1988). The results shown in Table 1 demonstrated, in the absence of complement, a neutralizing activity in the Hanti F 3205 sera and, although to a lower extent, in the Hanti HN 3208 sera. These data, thus, show that the VV3205 and VV3208 produce MuV F and MuV HN proteins, respectively, in vitro and in vivo. The recombinant proteins appear to be immunogenic for hamsters and are thus appropriately presented to the immune system.

Polyclonal sera derived from hamsters immunized with VV3205 and VV3208 were then used for protection experiments on newborn hamsters intracerebrally infected with the Kilham strain of MuV. This strain develops in these animals a lethal meningoencephalitis upon intracerebral (i.c.) inoculation and has been already used extensively in pathogenic studies of viral brain infections (Kilham & Overman, 1953; Merz & Wolinsky, 1981; Wolinsky et al., 1974). Thus, groups of 1-day-old hamsters were infected by i.c. inoculation with 10^4 p.f.u. of each virus and immunized 24 h later by intraperitoneal
injection with 100 μl of Hanti F 3205 or Hanti HN 3208 sera (see Table 1), having end-dilution ELISA titres of $8.2 \times 10^4$. The negative control group consisted of infected newborn hamsters immunized with 100 μl of Hanti β-galactosidase (βgal) serum. The survival of newborn hamsters was then followed for up to 16 days post infection. As seen in Fig. 2, the passive transfer of Hanti F 3205 or of Hanti HN 3208 resulted in a significant protection against MuV infection (72 and 86% respectively, 12 days post infection) whereas the transfer of Hanti βgal was completely non-protective since all animals were dead on day 12 post infection. In spite of a relatively high difference between the in vitro neutralizing capacity of Hanti F 3205 serum and that of Hanti HN 3208 serum, both antisera appear similarly efficient in terms of protection in vivo; it might be interesting to evaluate the combined effect of both antisera to see if complete protection of infected animals can be obtained.

Previous work (Löve et al., 1986; Wolinsky et al., 1985) has shown that MAbs raised against the F and HN proteins of the MuV Kilham strain are protective in necrotizing mumps meningoencephalitis. The present results not only confirm this observation, using polyclonal antibodies, but also indicate that recombinant versions of F and HN proteins adopt an appropriate conformation to elicit a neutralizing and protective immune response. In conclusion, the data support the choice of the F and HN MuV glycoproteins as targets for the development of a recombinant subunit vaccine for MuV.

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References


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