Virulence and pathogenesis of non-virulent and virulent strains of pseudorabies virus expressing envelope glycoprotein E1 of hog cholera virus


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Pseudorabies virus (PRV) expressing the envelope glycoprotein E1 (E1) of hog cholera virus (HCV) was used as a model to study the potential risks connected with the use of a live herpesvirus vaccine expressing a foreign gene. The gene encoding E1 was inserted into the glycoprotein X (gX) locus of both a virulent PRV strain and a non-virulent PRV strain in which the virulence genes encoding glycoprotein I (gI) and thymidine kinase (TK) had been inactivated. We investigated whether strain M205 (gI-,TK-,gX ,E1 +) had a changed cell or host tropism or virulence compared with strain M206 (gI , TK , gX ) in pigs, rabbits, hamsters, rats, mice and rhesus monkeys. The insertion of E1 into this non-virulent PRV strain caused no change in cell or host tropism. However, pigs inoculated with M205 shed less virus over a shorter period than pigs inoculated with M206. Theoretically, virulent PRV strains expressing E1 (gX-,E1 +) could arise through transfer of the E1 gene of M205 to a virulent PRV strain. Therefore, we inoculated pigs with strain M12 (gX-,E1 +) or the control strain M104 (gX-) and compared the virulence and pathogenesis. M12 and M104 were of approximately equal virulence and the pathogenesis of both strains was similar. We concluded that incorporating E1 of HCV into the gX locus of PRV did not change cell or host tropism, nor did it change the virulence of either non-virulent or virulent PRV.

Introduction

Recently, investigators constructed a live pseudorabies virus (PRV) vector that expresses the envelope glycoprotein E1 (E1) of hog cholera virus (HCV) (van Zijl et al., 1991). Immunization with this PRV vector protected pigs against both pseudorabies (Aujeszky’s disease) and hog cholera. The gene encoding E1 was inserted into the gX locus of a live attenuated PRV strain that was obtained by inactivating the glycoprotein I (gI) and thymidine kinase (TK) genes. The gI and TK proteins are important for virulence of PRV, but the gX gene seems to have no significant role in the virulence or immunogenicity of PRV (Thomsen et al., 1987; Kimman et al., 1992). This PRV strain, designated M205, was used as a model to study the risks of using a live herpesvirus expressing a foreign gene. Although some investigators have studied the biological properties of poxvirus vectors that express heterologous genes (Spehner et al., 1988; Perkus et al., 1989; Taylor et al., 1991), no one has examined the changes that might occur in the biological properties of herpesvirus vectors.

The pathogenesis of HCV and PRV differs; PRV affects the respiratory tract and the central nervous system, whereas HCV has a distinct affinity for cells of the lymphoreticular organs. For example, in persistently viraemic animals, infectious HCV can be isolated from blood mononuclear cells (van Oirschot, 1979, 1983). The level of viraemia is high and is observed frequently. In contrast, viraemia is observed only occasionally after infection with neurotropic PRV and then only at a low level (Wittmann et al., 1980). Although the role of E1 in the pathogenesis of HCV is unknown, Wensvoort et al. (1988) demonstrated that E1 induces a protective immune response and that protected pigs invariably have antibodies against E1. They were also able to prepare strongly neutralizing monoclonal antibodies directed against E1 (Wensvoort, 1989; Wensvoort et al., 1990). Therefore, E1 is probably involved in adsorption, or penetration of HCV into host cells.

Because changes in the interaction of a vector virus with host cells may be due to the presence of the foreign protein in the virus envelope, we used immunoelectron microscopy to investigate whether E1 was incorporated in the envelope of the M205 virions. We also investigated whether the expression of E1 affected cell, tissue or host...
tropism, or altered virulence. PRV strain M205 (gI-, TK-, gX-, E1+) or the control strain M206 (gI-, TK-, gX), without E1, were therefore inoculated into pigs (the natural host of PRV), rhesus monkeys and other PRV-susceptible animals such as rabbits, hamsters, rats and mice.

Finally, we tested whether it was possible that when the E1 gene of M205 was genetically exchanged for the gX gene of a virulent PRV strain, a strain of increased virulence or unusual pathogenesis would be obtained. Genetic exchanges between virulent or attenuated PRV strains have been reported to yield new virus strains more virulent than the parent strains (Henderson et al., 1990, 1991; Katz, 1990). Therefore, a 'worst case' gX-, E1+ recombinant virus (M12) and a gX- control strain which lacked E1 (M104) were constructed. Pigs were inoculated with these strains and the virulence and pathogenesis of both strains were compared.

Methods

Cells, media and virus titration. The swine kidney cell line SK6 (Kasza et al., 1971) was cultivated in culture medium, consisting of Dulbecco's MEM supplemented with 5% fetal calf serum, t-glutamine (0.3 mg/ml), and the antibiotics penicillin (90 units/ml), streptomycin (100 μg/ml) and fungizone (4-5 μg/ml) in a humidified CO₂ incubator at 37°C. Baby hamster kidney (BHK) cells were cultivated in Eagle's Basal medium supplemented with lactalbumin hydrolysatе (6 g/l), proteose peptone (4-5 g/l), tryptose phosphate (4-5 g/l), tryptone (4-5 g/l) and antibiotics as described above.

Virus multiplication after primary infection was measured by titrating oropharyngeal fluid (OPF) samples and organ suspensions on SK6 cells. Virus titres were expressed as log₁₀ p.f.u./g tissue or per g OPF.

Virus strains. The NIA-3 strain of PRV (McFerran & Dow, 1975) was used as the parent strain for the construction of mutant viruses. The construction and characterization of strain M205, the non-virulent vector expressing E1, and of the empty vector strain M206 have been described earlier (van Zijl et al., 1991).

Strain M12, the virulent vector expressing E1, was generated by in vivo overlap recombination of PRV wild-type DNA and the 5.1 kb EcoRV-NcoI fragment of plasmid pMZ-67 (van Zijl et al., 1991). This fragment contains the E1-coding information inserted in the gX locus and approximately 2 kb of adjacent PRV sequences. Recombinant virus was detected in an immunoperoxidase monolayer assay using anti-E1 monoclonal antibodies. It was plaque-purified twice and DNA restriction enzyme analysis was used to check for the absence of gross unintended mutations.

Strain M104 (gX-) was constructed by de Wind et al. (1990), using a linker insertion mutagenesis technique. This strain has an inserted oligonucleotide with translational stop codons in all three reading frames in the 5' region of the gX gene.

The non-virulent PRV strain Bartha (Bartha, 1961) has a deletion of 38 kb in the U₁ region of the genome and does not express gI, gp63, an 11K and a 28K protein. It also has deletions in the U₁ region that affect genes involved in the nucleocapsid assembly (Lomniczi et al., 1987).

Immunoelectron microscopy. BHK cells were infected with the virus M205 or with the PRV vaccine strain Bartha (Bartha, 1961) at an m.o.i. of 1. Twenty hours after virus infection, 50% of the cells had degenerated, but not yet lysed. The cells were fixed for 2 h at 0°C with 2% paraformaldehyde, 0.2% glutaraldehyde in 0.1 M phosphate buffer pH 7.5. Cells were pelleted at 100 g and washed twice with phosphate buffer. The cell pellet was dehydrated and embedded in Lowicryl K4M (Chiovetti, 1982).

Sections of 50 nm thickness were cut with a Reichert Jung Ultracut E microtome and were mounted on one-hole nickel grids (Veco) with a formvar 1595 E layer (Merck). The sections were immersed in 0.5% BSA in PBS for 10 min, drained, and immersed for 2 h at 37°C in dilutions in PBS containing 0.5% BSA of polyclonal rabbit sera containing antibodies directed against PRV or HCV. Owing to fixation, the reactivity of monoclonal antibodies directed against E1 was lost and therefore a polyclonal anti-HCV serum was used for this study. After being thoroughly washed with a jet of PBS and then drained, the sections were placed on 20 nm gold-labelled goat anti-rabbit IgG conjugate (Geoghegan & Ackerman, 1977) for 30 min at room temperature. After being washed with PBS and distilled water, the sections were dried overnight, stained with uranyl acetate and lead citrate and were examined in a Philips CM10 electron microscope. The average number of gold particles attached to the envelope per virion section was calculated by counting the gold particles adherent to a sample of 100 virions.

Animals. Three-week-old Dutch Landrace pigs were obtained from the specific pathogen-free (SPF) herd of the Central Veterinary Institute. The pigs were born to unvaccinated sows and had no antibodies against PRV or HCV before the start of the experiments. Pigs of different litters were randomly assigned to experimental groups. The pigs had access to food and water ad libitum during the whole day. SPF mice, rats, hamsters and rabbits were obtained from Charles River (Wiga). Six-week-old BALB/c mice, 6-week-old Wistar (outbred) rats, 6-week-old Syrian gold hamsters and 4-month-old New Zealand rabbits were used. Two- to three-year-old rhesus monkeys (Macaca mulatta) were bred and kept at the TNO Primate Center in the Netherlands. These animals had no herpesvirus B antibodies and were negative for tuberculosis reaction. All animals were randomly assigned to experimental groups. During the experiments each group was housed in separate isolation rooms.

Collection of samples. Swabs were used to collect OPF samples from pigs once daily from 1 day before until 11 days after inoculation. Swabs were extracted with 4 ml of culture medium supplemented with the antibiotics polymyxin B (50 μg/ml) and kanamycin (100 μg/ml). The total weight of the collected OPF was measured after centrifugation of the swab in a special container. The daily mean ±S.D. of virus excretion (log₁₀ p.f.u./g OPF) for the 11 day period after inoculation was compared with that of pigs inoculated with M205 or M206. Student's t-test was used to evaluate the results statistically.

After inoculation, blood was collected three times a week in tubes containing EDTA. Virus was isolated from peripheral blood leukocytes (PBLs) by first mixing the blood with a double quantity of 0.83 % ammonium chloride to lyse the erythrocytes. After 20 min incubation at room temperature, the PBLs were pelleted, washed three times with PBS pH 7.4 and resuspended in the original blood volume with culture medium. After being frozen and thawed, PBL suspensions were titrated on SK6 cells.

An Analysis Instruments AB 134 cell counter was used to determine the number of leukocytes, thrombocytes and crythrocytes in blood samples.

Serum was collected once a week and tested for neutralizing antibodies against PRV as described by de Leeuw et al. (1982). Various tissues (Table 1) were collected from pigs that died or were killed for virological or pathological examination.

Virological and pathological examination. After autopsy, 10% (w/v) tissue suspensions were made in culture medium supplemented with the
antibiotics polymyxin (50 μg/ml) and kanamycin (100 μg/ml), and the suspensions were titrated on SK6 cells. Tissues for histological examination were fixed in 4% buffered formalin pH 7.4, dehydrated with alcohol and acetone, and embedded in paraffin. Sections (6 μm thick) were stained with haematoxylin. Viral antigen in tissues was detected with an indirect immunoperoxidase assay (Pol et al., 1991) using a polyclonal rabbit serum directed against PRV.

Clinical signs of disease. The pigs were observed once a day for clinical signs. Rectal temperatures were measured once daily until 11 days after inoculation. The animals were weighed three times a week for a month to detect possible growth retardation. Clinical signs of Aujeszky’s disease were monitored as described by Kimman et al. (1992). Briefly, fever was defined as a rectal temperature above 40 °C. Respiratory signs were defined as nasal discharge, sneezing, coughing and forced respiration. Neurological signs were defined as scratching, ataxia, vomiting, paralysis, tremor and convulsions.

Experimental design

Experiment 1. Twenty pigs were divided into two groups. The pigs were inoculated both intranasally (0.5 ml per nostril, slowly during inspiration) and intravenously with 1 ml containing 10^7 p.f.u. of strain M205 or with 1 ml containing 10^7 p.f.u. of the vector strain M206. OPF and blood samples were collected as described above. Five days after infection, five pigs of each group were killed and various tissues were collected for virological and pathological examination.

Experiment 2. Ten rabbits, hamsters, rats, and mice were divided into two groups. The animals were subcutaneously inoculated with either 1 ml containing 10^5 p.f.u. of strain M205 or with 1 ml containing 10^6 p.f.u. of the vector strain M206. All animals were also intranasally inoculated with these PRV mutants. The virus quantity that could be administered intranasally depended on the species. Mice were intranasally inoculated with 5 × 10^6 p.f.u., rats with 25 × 10^4 p.f.u., hamsters with 2 × 10^6 p.f.u. and rabbits with 50 × 10^4 p.f.u. Two uninoculated hamsters, rats and mice were kept separate as controls. Twice a day the animals were observed for clinical signs of any kind. Tissues were collected from animals that died during the experiment for virological and pathological examination. One month after inoculation, serum was collected from the surviving animals and tested for neutralizing antibodies.

Experiment 3. Six seronegative rhesus monkeys were divided into two groups. Four were inoculated intranasally with 10^5 p.f.u. of strain M205 and two with 10^6 p.f.u. of the vector strain M206. The monkeys were observed daily for clinical signs. After 3 weeks, serum was collected and tested for neutralizing antibodies.

Experiment 4. Ten pigs were divided into two groups and housed in positive-pressure isolation boxes. All infection and sampling procedures were performed in the isolation boxes. The pigs were inoculated intranasally (see experiment 1) with 1 ml 10^6 p.f.u. of gX^-E1+ mutant M12 or with 1 ml 10^5 p.f.u. of the gX^- mutant M104. Twice daily the pigs were observed for clinical signs. Tissues were collected from animals that died during the experiment or were killed at the end for virological and pathological examination.

Results

Immunoelectron microscopic examinations

Changes in the interaction of a vector virus with host cells might be caused by the presence of the foreign protein in the envelope of the vector. To investigate whether E1 was present in the envelope of vector M205 (TK+,gI-,gX+,E1+), we used immunoelectron microscopy to study BHK cells infected with M205 or the PRV vaccine strain Bartha, as a negative control. Infected cells were stained with polyclonal sera directed against HCV or PRV. In cell cultures infected with M205, the envelope of virions released from the cells showed some labelling with the anti-HCV serum and more with the anti-PRV serum (Fig. 1 a, b). The average number of gold particles per M205 virion envelope detected by the anti-HCV serum was 1.4 (±1.2). In contrast, in cell cultures infected with the Bartha strain, the envelope of virions released from the cells showed no labelling with anti-HCV serum. The average number of gold particles in the envelope of M205 virions detected by the anti-PRV serum was 4.4 (±0.7). The results indicated that E1 of HCV is indeed present on the envelope of the PRV vector.

Fig. 1. Immunoelectron micrographs of BHK cells infected with M205 or the PRV vector M205 (gI+,TK+,gX+,E1+) carrying the envelope glycoprotein E1 of HCV. Labelling in virions released from the cell was detected in the envelope of M205 virions. Infected cells were stained with a polyclonal serum directed against HCV (a) or with a polyclonal serum directed against PRV (b). Bar markers represent 200 nm.
M205. These results agree with the findings of R. J. M. Moormann et al. (unpublished results) who also found small amounts of E1 present on the envelope of M205 virions, using immunoprecipitation techniques.

Virulence and pathogenesis of M205 and M206 in pigs

The purpose of the experiment was to investigate whether the expression of HCV E1 by M205 affects cell or tissue tropism or whether it changes the virulence of the PRV vector for pigs. Three-week-old pigs were inoculated both intranasally and intravenously with $10^7$ p.f.u. of the virus M205 or the control vector M206. Both strains replicated (see below) and induced neutralizing antibodies; titres peaked within 2 weeks after infection (data not shown). The infection did not cause any clinical signs of disease. Rectal temperatures remained below 40 °C and the pigs did not lose weight. The number of thrombocytes, erythrocytes and leukocytes did not differ significantly between pigs infected with M205 or M206 (Fig. 2). Furthermore, no histopathological lesions resulted from either strain and no viral antigen was detected in paraffin sections of tissues collected 5 days after inoculation.

Virus shedding in OPF, tissues and PBLs of pigs inoculated with M205 and M206

Virus shedding in OPF differed significantly between the two groups of pigs. The daily mean virus excretion (± s.d. in log_{10} p.f.u./g OPF) from day 1 to day 9 after inoculation of pigs inoculated with M205 was 0.9 (±0.6), whereas the excretion of pigs inoculated with M206 was 2.0 (±0.7) (Fig. 3). Thus, pigs shed significantly ($P < 0.05$; Student's $t$-test) less virus after inoculation with M205 than pigs inoculated with M206. Moreover, pigs inoculated with M205 shed virus for a shorter period than those inoculated with M206. The mean number of days of virus excretion (± s.d.) of pigs inoculated with M205 was 3.2 (±1.8), whereas animals that received M206 excreted virus for 6.0 (±2.1) days. The difference in time ($t = 2.26$) was just below the level of significance ($t > 2.31$ for $P < 0.05$).

At day 5 post-inoculation, five pigs in each group were killed. Small quantities of virus were recovered from pharyngeal mucosa, tonsils, mandibular lymph node and adrenal glands. No virus, however, was recovered from nervous tissues. Virus was recovered from the nasal mucosa and the retropharyngeal lymph node of pigs inoculated with M205 and from the subparotideal lymph node and the lungs of pigs inoculated with M206 (Table 1). This variation was not considered significant because the virus titres were low and may be due to normal biological variation among pigs. Finally, no virus was recovered from PBLs in either group.

Virulence and pathogenesis of M205 and M206 in hamsters, rabbits, rats and mice

The purpose of the experiment was to investigate whether the expression of E1 changes the pathogenesis or virulence of the vector PRV in other species. Hamsters, rats and mice did not develop signs of disease after intranasal and subcutaneous inoculation with either M205 or M206. A few mice showed local scratching for 1 day at the site of inoculation. This behaviour was probably caused by a local infection. None of the hamsters, rats or mice developed any clinical signs.

Rabbits, however, appeared susceptible to both
PRV strains expressing glycoprotein E1 of HCV

Three of five rabbits died after infection with M205 and four of five rabbits died after infection with M206. The rabbits developed characteristic signs of Aujeszky's disease such as ataxia, scratching, neurological signs and paralysis, and in addition breathed rapidly. Virus was recovered from nasal mucosa, cerebral pons and trigeminal ganglia of dead rabbits (data not shown).

Neutralizing antibodies were detected in all surviving rabbits, 80% of hamsters, 50% of rats and 10% of mice. Neutralizing antibody titres did not differ significantly between animals inoculated with either strain.

**Virulence and pathogenesis of M205 and M206 in rhesus monkeys**

Four rhesus monkeys were intranasally inoculated with \(10^6\) p.f.u. of M205 and two were inoculated with \(10^6\) p.f.u. of M206. The rhesus monkeys did not develop any clinical signs or neutralizing antibodies against PRV or HCV.

**Virulence and pathogenesis of mutants M12 and M104 in pigs**

Because genetic exchange could occur between the vector strain M205 and wild-type PRV, yielding a virulent strain (gI\(^+\), TK\(^+\)) that expresses E1, we constructed such a 'worst case' mutant. Three-week-old pigs intranasally inoculated with \(10^6\) p.f.u. of this mutant M12 (gX\(^-\), E1\(^+\)) or with the control mutant M104 (gX\(^-\)) developed severe signs of Aujeszky's disease. From 2 days after inoculation, both groups developed fever. Both strains seemed about equally virulent and caused severe neurological signs such as ataxia, tremor, convulsions and paralysis. Pigs inoculated with M104 developed these symptoms about 1 day earlier than pigs inoculated with M12. All five pigs died of infection with M104, and three of five pigs died of infection with M12; the other two developed less severe neurological signs and recovered. The three others that died had a longer mean time to death (8.7 days) than did the five pigs that died of infection with M104 (6.2 days).

Both viruses caused lesions of the bulbus olfactorius and trigeminal ganglia with some degeneration and necrosis of neurons. Degeneration and necrosis of neurons was accompanied by neuronophagia, perineuronal gliosis and mononuclear inflammatory infiltrates. Viral antigen was detected in the bulbus olfactorius of two pigs inoculated with M12 (Fig. 4a) and in three pigs inoculated with M104. Furthermore, viral antigen was detected in the trigeminal ganglia of two pigs inoculated with M104 (Fig. 4b).

**Table 1. Virus content in various tissues of 3-week-old pigs after inoculation with four PRV strains**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>M206</th>
<th>M205</th>
<th>M104</th>
<th>M12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal mucosa</td>
<td>-</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Pharyngeal mucosa</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Tonsil</td>
<td>0.6</td>
<td>0.9</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Subparotideal l.n.</td>
<td>0.6</td>
<td>0.8</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Mandibular l.n.</td>
<td>0.2</td>
<td>0.2</td>
<td>0.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Retropharyngeal l.n.</td>
<td>0.6</td>
<td>0.6</td>
<td>0.7</td>
<td>2.9</td>
</tr>
<tr>
<td>Lung</td>
<td>0.9</td>
<td>1.4</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Heart</td>
<td>0.5</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.2</td>
<td>0.5</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>0.1</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulbus olfactorius</td>
<td>4.4</td>
<td>2.9</td>
<td>4.8</td>
<td>2.5</td>
</tr>
<tr>
<td>Pons cerebri</td>
<td>2.7</td>
<td>2.0</td>
<td></td>
<td>1.7</td>
</tr>
<tr>
<td>Medulla oblongata</td>
<td>2.3</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>2.3</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trigeminal ganglion</td>
<td>3.9</td>
<td>2.7</td>
<td></td>
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</tbody>
</table>

* Five pigs of experiment 1 were killed and tissues were collected on day 5 p.i.
† Five pigs of experiment 4. Tissues were collected when pigs died on days 5 to 8 p.i.
‡ Three animals of experiment 4. Tissues were collected when pigs died on days 6 to 11 p.i.
§ No virus recovered from the tissue.
| l.n., Lymph node. |
nodes of the oropharyngeal region. Thus, in contrast with the pigs inoculated with the gI-, TK- mutant strains, M12 and M104 infected the central nervous system and multiplied to higher titres. Virus titres were somewhat higher in oropharyngeal and brain tissues of pigs inoculated with M104 than in those infected with M12 (Table 1). In only one pig, which died 12 days after infection with M12, no virus was recovered from the brain tissues. Nonetheless, inflammatory lesions and perivascular cuffing were still observed in sections of brain tissues of this pig, probably indicating that the virus had already been cleared.

Discussion

This study is the first to evaluate potential changes in the biological behaviour of a live herpesvirus vector through the incorporation of a foreign gene. Pigs (natural host), rabbits, hamsters, rats, mice and rhesus monkeys were inoculated with a non-virulent PRV vector expressing E1 of HCV.

During assembly, PRV envelope proteins are routed from the endoplasmic reticulum through the Golgi complex to the plasma membrane. In the Golgi complex the glycoproteins are incorporated into the viral envelope during budding of the virus (Whealy et al., 1991). Vector PRV carrying E1 may have an altered host range if E1 also functions as a surface or attachment protein in the vector virus. We performed immunoelectron microscopic studies to investigate whether E1 was associated with the envelope of the PRV vector. The envelope of M205 virions released from the cell indeed showed some labelling. Intracellular labelling of the E1 protein in M205 virions was confined to the endoplasmic reticulum and the Golgi complex. Thus, this study indicated that E1 is processed in the endoplasmic reticulum and the Golgi complex and incorporated in the PRV vector.

Until now, there has been scant evidence that expression of foreign genes in carrier viruses can alter cell tropism or virulence. For example, Kost et al. (1989) found that vaccinia virus, expressing the envelope glycoprotein I of PRV, was more virulent for mice than wild-type vaccinia virus after intracranial inoculation. However, we found no changes in virulence, cell or tissue tropism of the PRV vector carrying E1. Expression of E1 of HCV did not increase the infectivity of the virus for leukocytes. Pigs inoculated with M205 or M206 had no significant differences in the number of PBLs. The number of leukocytes in pigs infected with M205 varied within the limits considered normal for pigs of 3 to 5 weeks old. The number of erythrocytes and thrombocytes decreased slightly immediately after infection during the acute phase of infection, but returned to normal after 4 days. This is probably an indication of an acute phase response. However, because uninfected controls were not included, the changes can not be ascribed to an acute phase response with certainty. At present we are investigating infection by M205, M206, M12, M104 and other PRV mutants of swine peripheral blood mononuclear cells in vitro. Preliminary results of these studies (unpublished) are consistent with the findings described above, namely that the incorporation of the E1 protein of HCV in the PRV vector does not alter the replication of PRV in swine PBLs.

In the oropharyngeal area, replication of the PRV vector carrying E1 was reduced. E1 inserted into the gX locus of the PRV gene may have been a replicative disadvantage for the PRV vector. Because the E1 protein is normally incorporated in membranes of the endoplasmic reticulum in HCV-infected cells (Hulst et al., 1993), its expression in M205- and M12-infected cells could interfere with the processing or incorporation of

Fig. 4. Porcine nervous tissues infected with M12 or with M104. Viral antigen (dark stain) in sections was detected by the immunoperoxidase method. (a) Neurons in the bulbus olfactorius infected with M12. The neurons of the mitral cell layer are mainly infected. (b) Trigeminal ganglion infected with M104. Large neurons are infected. Degeneration of neurons was accompanied by inflammatory infiltrates. Bar markers represent 50 μm.
PRV glycoproteins and therefore reduce the replication rate of M205 and of M12. The vector virus carrying E1 may also be less stable.

To investigate possible changes in virulence for different hosts, we also inoculated M205 and M206 into rabbits, hamsters, rats, mice and rhesus monkeys. All surviving rabbits, 80% of the hamsters, 50% of the rats, 10% of the mice and none of the rhesus monkeys developed neutralizing antibodies. Only rabbits developed clinical signs and most died from the infection. In other animal species, strain M205 was no more virulent than strain M206.

A potential risk of using live vector viruses is the exchange of genetic information between the vector strain and other vaccine or wild-type strains. Homologous or illegitimate recombination between the vector virus and the field virus might yield a virulent mutant carrying a foreign gene which may therefore have changed biological properties (Kimman, 1992). Herpesviruses can cause latent infections predominantly in the trigeminal ganglia and in brain tissues, but also in tonsils, lymphocytes and lungs (Rziha et al., 1982). The persistence of herpesviruses in the host might increase the chance of genetic exchange. The consequences of such an exchange depend on the site in which the foreign gene is inserted in the vector virus. When the gene is inserted in a viral gene that contributes to virulence (e.g. TK and gI of PRV), then recombination with the field virus will probably yield a less virulent virus. However, when the heterologous gene is inserted in a viral gene that does not contribute to virulence (e.g. the gX gene of PRV), a virulent mutant could theoretically develop. Such a strain could have the transmission properties of the virulent strain and might even be better able to survive than wild-type strains. We constructed such a 'worst case' mutant, referred to as M12 (gX+ E1+). M12 was virulent for pigs, although its virulence may be slightly reduced compared to the gX− control mutant M104. Two pigs inoculated with M12 survived the infection, whereas all five pigs died after inoculation with M104. The pigs that died after infection with M12 had a longer mean time to death than those inoculated with M104. Finally, virus titres in tissues from pigs infected with M12 were somewhat lower than virus titres in tissues of pigs inoculated with M104. Again, these findings indicate that incorporation of E1 may have reduced the replication of vector PRV. Strain M104 was highly virulent for pigs. Thus, this experiment confirmed that gX is not essential for virulence of PRV and that insertion of a foreign gene in the gX locus only slightly reduced virulence.

We conclude that the expression of E1 in the gX locus of PRV does not change cell or host tropism, nor does it apparently increase or drastically decrease the virulence of the PRV vectors. It did, however, reduce virus replication in the oropharyngeal region. The E1 protein expressed by PRV probably interferes with the processing of PRV glycoproteins.

References


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