Dissemination of wild-type and gC-, gE- and gI-deleted mutants of Aujeszky’s disease virus in the maxillary nerve and trigeminal ganglion of pigs after intranasal inoculation

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Aujeszky’s disease virus (ADV) is a well known neurotropic virus in pigs. In the present study the mechanism of spread of ADV along the maxillary nerve and the role of the viral envelope glycoproteins gC, gE and gI in this process was examined in pigs. The Ka parental strain of ADV and its gC-, gE- and gI-deleted mutants were inoculated intranasally in pigs, after which virus dissemination in the maxillary nerve and the trigeminal ganglion was monitored at time intervals by means of virus isolation. The parental strain was isolated from both the nasal mucosa and the trigeminal ganglion at 21 h post-inoculation (p.i.), whereas the middle part of the connecting maxillary nerve was positive only after 48 h p.i. It appears, therefore, that ADV travels from the nasal mucosa via the nerve towards the ganglion in a non-infectious form, and then replicates in the neuronal somas, after which infectious virus is transported towards the nasal mucosa. Although all mutants were present at 48 h p.i. in the nasal mucosa and the trigeminal ganglion, the appearance of infectious virus in the maxillary nerve was clearly delayed with the gE- and gI mutants. It is suggested that glycoproteins gE and gI are involved in the axonal transport of infectious ADV away from neuronal cell bodies, also called anterograde transport.

Aujeszky's disease virus (ADV), a porcine herpesvirus, is its ability to cause nervous disorders in neonatal pigs. After uptake and primary replication in the upper respiratory tract, ADV spreads towards the central nervous system (CNS) by means of one or more of the cranial nerves such as the olfactory and trigeminal nerves (McFerran & Dow, 1965; Sabo et al., 1969; Wittmann et al., 1980).

It is generally accepted that the neuron plays an important role in neural invasion by herpesviruses such as ADV and herpes simplex virus type 1 (HSV-1) (Cook & Stevens, 1973; Field & Hill, 1974; Hill et al., 1972; Kristensson et al., 1971; McCracken et al., 1973; Wildy, 1967). The replication cycle of herpesviruses in neurons has been studied most frequently with HSV-1 in rat or human neuronal cell cultures (Lycke et al., 1984, 1988). These studies have shown that the virus enters into axonal extensions by fusion of the virus envelope with the neuronal cell membrane, after which nucleocapsids are transported towards the nerve cell body. After formation of viral components and assembly in the nerve cell bodies, newly formed enveloped particles are transported in the axons towards the synaptic terminals (Lycke et al., 1984, 1988). Thus, considering the nerve cell body as the basic element of the neuron, two directions of virus axonal transport can be put forward: transport of capsids towards the nerve cell body, called retrograde transport, and transport of enveloped particles away from the nerve cell body, called anterograde transport.

Information regarding in vivo transport of herpesviruses has been derived by studying the kinetics of dissemination of infectious HSV-1 in spinal nerves and their corresponding spinal ganglia (Cook & Stevens, 1973; Hill et al., 1972; Klein & DeStefano, 1983; Kristensson et al., 1971; Wildy, 1967). The ganglion contains the neural cell bodies and the nerves their extensions. In such a study, Klein & DeStefano (1983) showed that after inoculation of HSV-1 in the footpad of mice, virus travels retrogradely in the sciatic nerve towards the lumbosacral ganglia in a non-infectious form. After replication in the nerve cell bodies of the ganglia, HSV-1 is transported anterogradely in an infectious form towards the inoculation site. Studies
which aim to examine transport of ADV in the neurons of the pig have not been performed so far.

Because transport along a chain of neurons is necessary for the spread of the virus to the depths of the CNS, it is apparent that the more effective neuronal transport is, the higher the neuroinvasive capacity will be. Previous studies have shown that effective invasion by ADV of the CNS via the olfactory and trigeminal nervous pathways of pigs is dependent on the presence of the non-essential envelope glycoproteins gI and particularly gC, whereas envelope glycoprotein gE is dispensable for such a spread (Kritas et al., 1994a, b). However, there are no reports on whether the functions of those glycoproteins in neuroinvasion are associated with transport of ADV in the neurons. In the present study, therefore, we examined the dissemination of an undeleted ADV strain and its gC−, gE− and gI− mutants from the nasal mucosa to the trigeminal ganglion via the maxillary nerve in the pig during the acute phase of infection. The maxillary nerve was selected for such an examination because it provides the main innervation of the nasal mucosa (Fig. 1) and because it is long enough to allow several pieces of this nerve to be sequentially examined. This sensory nerve contains peripheral neuron extensions of the pseudounipolar neuronal cell bodies which are located in the trigeminal ganglion.

A total of 17 piglets that lacked antibodies against ADV were inoculated intranasally at the age of 7 days with the virulent Ka strain of ADV or one of its genetically engineered derivatives, Ka gC−, Ka gE− or Ka gI−. A 1 ml volume (0.5 ml per nostril) of virus suspension containing 10^3 TCID_{50} was slowly administered by means of a catheter. All ADV strains were kindly supplied by T. C. Mettenleiter (Insel Riems, Germany). The Ka parental strain is of uncertain origin and is virulent for newborn pigs (Kaplan & Vatter, 1959). For the construction of mutants the DNA was cleaved with BstEII and SphI in order to delete a large part of the gE gene, with StuI and NcoI in order to delete a large part of the gI gene, or with XhoI–XhoI in order to delete a large part of the gC gene (Mettenleiter et al., 1987, 1988).

Six piglets were inoculated intranasally with the Ka parental strain, four piglets with the Ka gE− mutant, four piglets with the Ka gI− mutant and three piglets with the Ka gC− mutant. The piglets were killed at various times post-inoculation (p.i.) as indicated in Table 1. The nasal mucosa, maxillary nerve and trigeminal ganglion were collected and processed for virus isolation and quantification. For some pigs, samples were collected from both the left and right side of the body. After removal of the lateral orbital wall and eyeball, the maxillary nerve was located and was sectioned between the foramen orbitotorotundum and its entrance in the spheno-palatine and infraorbital forams. Some maxillary nerves were cut into three pieces (Fig. 1): one close to the periphery (distal part), one close to the trigeminal ganglion (proximal part) and one in the middle (mid part). The cerebellum and cerebrum of all pigs were also collected and processed for virus isolation. Methods used for virus isolations and titrations have been described previously (Kritas et al., 1994b). Briefly, 10% suspensions of maxillary nerves and trigeminal ganglia, and 20% suspensions of other tissue samples were made and centrifuged. Tenfold dilutions of the supernatants were inoculated on swine testicle (ST) cell culture. The cell cultures were observed for a period of 5 days for cytopathic effect.

The results of virus isolation and titration are presented in Table 1. The Ka parental strain was isolated from the nasal mucosa and the trigeminal ganglion as early as 21 h p.i. However, no infectious virus was detected in the mid part of the maxillary nerve at 21 h and 29 h p.i. The absence of virus in the mid part of the maxillary nerve can be explained by the fact that virus is transported via the maxillary nerve towards the trigeminal ganglion in a non-infectious form. Although this is assumed to be the most probable explanation, the possibility that small, undetectable amounts of infectious virus are passing through cannot be excluded. Another explanation could be that virus reached the trigeminal ganglion via the blood. However, under such circumstances virus would also be expected to be isolated from...
Table 1. Virus quantification in peripheral tissues of the trigeminal pathway

<table>
<thead>
<tr>
<th>Pig no.</th>
<th>Strain inoculated</th>
<th>Killed (h p.i.)</th>
<th>Side of body*</th>
<th>Nasal mucosa</th>
<th>Maxillary nerve</th>
<th>Trigeminal ganglion</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>Ka parental</td>
<td>21</td>
<td>L</td>
<td>5.3</td>
<td>Neg.†</td>
<td>Neg.</td>
</tr>
<tr>
<td>32</td>
<td>Ka parental</td>
<td>29</td>
<td>R</td>
<td>NA</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>33</td>
<td>Ka gC-</td>
<td>48</td>
<td>L</td>
<td>6.7</td>
<td>2.0</td>
<td>Neg.</td>
</tr>
<tr>
<td>34</td>
<td>Ka gC-</td>
<td>48</td>
<td>R</td>
<td>NA</td>
<td>5.2</td>
<td>Neg.</td>
</tr>
<tr>
<td>35</td>
<td>Ka gC-</td>
<td>96</td>
<td>L</td>
<td>7.2</td>
<td>5.7</td>
<td>4.5</td>
</tr>
<tr>
<td>36</td>
<td>Ka gC-</td>
<td>108</td>
<td>L</td>
<td>6.3</td>
<td>3.9</td>
<td>5.5</td>
</tr>
<tr>
<td>51</td>
<td>Ka gC-</td>
<td>48</td>
<td>L</td>
<td>5.2</td>
<td>2.4</td>
<td>3.8</td>
</tr>
<tr>
<td>52</td>
<td>Ka gC-</td>
<td>72</td>
<td>L</td>
<td>6.0</td>
<td>3.9</td>
<td>4.5</td>
</tr>
<tr>
<td>53</td>
<td>Ka gI-</td>
<td>108</td>
<td>L</td>
<td>5.5</td>
<td>5.5</td>
<td>3.4</td>
</tr>
<tr>
<td>71</td>
<td>Ka gI-</td>
<td>48</td>
<td>L</td>
<td>5.4</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>72</td>
<td>Ka gI-</td>
<td>72</td>
<td>L</td>
<td>5.7</td>
<td>Neg.</td>
<td>3.4</td>
</tr>
<tr>
<td>73</td>
<td>Ka gI-</td>
<td>120</td>
<td>R</td>
<td>6.0</td>
<td>3.8</td>
<td>4.0</td>
</tr>
<tr>
<td>74</td>
<td>Ka gI-</td>
<td>120</td>
<td>R</td>
<td>7.2</td>
<td>3.9</td>
<td>4.0</td>
</tr>
<tr>
<td>41</td>
<td>Ka gE-</td>
<td>48</td>
<td>L</td>
<td>5.7</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>42</td>
<td>Ka gE-</td>
<td>48</td>
<td>L</td>
<td>5.9</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>43</td>
<td>Ka gE-</td>
<td>120</td>
<td>R</td>
<td>NA</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>44</td>
<td>Ka gE-</td>
<td>130</td>
<td>L</td>
<td>5.5</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
</tbody>
</table>

* Left (L) and right (R) side of the body.
† Negative: sensitivity limit of assays was 10^1.0 TCID<sub>50</sub>/g tissue.
‡ The three parts of the maxillary nerve were pooled.
§ This sample was positive for viral antigens by immunofluorescence (method not presented here).
NA, Not available.

other neural tissues, which was not the case. Our attempts to isolate virus from the cerebellum and cerebrum at 21 h and 29 h p.i. were unsuccessful (data not shown), indicating that transport of the virus via the blood is unlikely. Also, another study has shown that viramia with ADV occurs in only 25% of oronasally infected pigs at 24 h p.i. (Nauwynck & Pensaert, 1995), so that the virus could hardly have reached both trigeminal ganglia via the blood in all pigs at 21 h and 29 h p.i. The absence of HSV in the mid part of the spinal nerves, while it was present in the corresponding ganglia, has also been reported (Klein & DeStefano, 1983). These authors suggested that HSV-1 travels in the nerves towards the ganglia in a non-infectious form. A similar process may occur with ADV in the maxillary nerve. As shown in Table 1 infectious parental Ka virus was found in the mid part of the maxillary nerve starting at 48 h p.i., with virus titres being lower than in proximal parts. These findings indicate that ADV first replicates in the trigeminal ganglion and then travels from the ganglion back to the nasal mucosa, but now in an infectious form.

All Ka mutants were detected in the nasal mucosa as early as 48 h p.i. (Table 1). The Ka gC- mutant showed a dissemination pattern resembling that of the parental strain. It was isolated from the trigeminal ganglion and all parts of the maxillary nerve, with titres being lower in the mid parts compared to the proximal parts. The lower titres obtained with this mutant compared to the parental strain are probably not due to lower virus production in the neural tissues but may reflect the less efficient detection of this mutant in cell cultures due to its defective adsorption, as explained previously (Kritas et al., 1994b).

Both gI- and gE- mutants were detected in the nasal mucosa and trigeminal ganglion at 48 h p.i., at which time these viruses were not isolated from the maxillary nerve. The Ka gI- strain was first detected in the mid part of the maxillary nerve at 72 h p.i., at virus titres lower than those in the proximal parts. The gE- mutant was not detected at any time from any part of the nerve. Furthermore, no virus was isolated from the cerebellum and cerebrum of pigs numbered 71, 72, 41, 42, 43 and 44 (data not shown in Table 1). These findings indicate that the gI- and gE- mutants were transported towards the trigeminal ganglion where local replication occurred, but that subsequent transport of these mutants (particularly gE-) towards the nasal mucosa and inner central nervous tissue was defective. Thus although gE and gI are not essential for entry of ADV into the nerve endings and replication in the neuronal cell body, they play a very
important role in the anterograde transport of virus which follows.

Observations with HSV-1 in neuronal cell cultures have shown that after virus enters neuronal extensions by fusion, the nucleocapsids are transported in a retrograde manner towards the nerve cell body; the virus then replicates in the nerve cell bodies and newly formed enveloped particles are transported in an anterograde manner towards the periphery (Lycke et al., 1984, 1988). A similar mechanism of neural spread probably occurs with ADV. It is transported towards the nerve bodies in the trigeminal ganglion in a non-infectious form (nucleocapsids) and, after replication, infectious enveloped virus particles are transported anterogradely from the ganglia towards the nasal mucosa. Since deletion of genes coding for gC, gE or gI alters the envelope of ADV but not the nucleocapsids, it is expected that retrograde transport of mutants which lack these glycoproteins will not be markedly affected when compared to the parental strain. Indeed, the isolation of all Ka mutants from the trigeminal ganglion soon after inoculation indicates that this was the case. In contrast, anterograde transport of newly formed enveloped particles of gI– and particularly gE– mutants from the trigeminal ganglion towards the maxillary nerve was clearly defective. How gI and gE modulate transport in the latter is completely unknown at present.

As similar anterograde transports of ADV may occur from the neuronal bodies of trigeminal ganglia towards the pons-medulla and from the trigeminal nuclei of the pons-medulla towards the thalamus, deletions of gE and gI may also affect them. A series of such defective anterograde transports along neuronal extensions may explain the less invasive character of gE– and gI– mutants in the CNS as shown previously (Kritas et al., 1994a).

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References


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