Comparison of neuropathogenicity of poliovirus in two transgenic mouse strains expressing human poliovirus receptor with different distribution patterns

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In order to determine the influence of poliovirus receptor (PVR) expression on poliovirus cell tropism and neuropathogenesis, two transgenic (tg) mouse models were produced in which PVR was expressed under the transcriptional control of the human PVR gene promoter (hg–PVR mice) and the CAG promoter (CAG–PVR mice). Then the pathogenicity of poliovirus after intracerebral inoculation of the type 1 Mahoney strain was compared. These showed completely different clinical and pathological changes. In the former, the expression of PVR in neurons in the central nervous system (CNS) conferred susceptibility to poliovirus, and a paralytic disease that resembled the human poliomyelitis occurred. In the latter, PVR expression was detected in glial and ependymal cells in addition to the neurons. Paralysis of the limbs and death were rarely observed and mice survived without showing substantial clinical abnormality. Histopathological examination revealed that glial and ependymal cells also became susceptible to poliovirus infection. Poliovirus antigens were mainly detected in ependymal and glial cells and hippocampal neurons near the lateral ventricles in the brain, but were not frequently detected in neurons in the brainstem unlike in the hg–PVR mice. The levels of viral antigens and virus recovered from the CNS of CAG–PVR mice began to decrease as early as 2 days after inoculation, which suggested induction of a fast immune response. These results suggest that the neuropathogenicity of poliovirus changes markedly depending on the specific expression of the PVR molecule in the CNS.

Introduction
Poliovirus (PV), which belongs to the Enterovirus genus of the family Picornaviridae (Rueckert, 1996), causes acute diseases of the central nervous system (CNS), such as aseptic meningitis, encephalitis and poliomyelitis in humans (Bodian, 1955; Sabin, 1956). Approximately less than one out of every hundred people naturally infected with wild-type PV develop a paralytic disease. Motor neurons in the anterior horn of the spinal cord and neurons in the brainstem are highly susceptible to PV and are destroyed by lytic replication of PV. The destruction of the motor neurons causes flaccid paralysis of the limbs. Inflammatory responses such as neuronophagia and perivascular cuffing are observed in the parenchyma of the CNS. Infection of the glial and ependymal cells is not observed. This distinct tropism of PV for neurons is evident when compared with other viruses. Some viruses are also neurovirulent in acute infection but invade mainly non-parenchymal sites of the CNS. For example, the most frequent CNS disease caused by mumps virus is meningitis (Wolinsky, 1996). Inflammations are, in most cases, limited to the choroid plexus, ependyma and meninges. Almost all coxsackieviruses (CVs) of both A and B groups (CVA and CVB), as well as most echoviruses, have been associated only with meningitis and seldom with severe neurological diseases, including paralysis and severe sequelae (Johnson, 1998; Melnick, 1996). CVs and echoviruses do not
frequently invade parenchymal tissues of the CNS except in newborns. These observations have led us to consider that the distinct pathogenic property of PV in the CNS is, at least in part, controlled by its strict tropism to the neurons.

The poxvirus receptor (PVR) has been considered as a major determinant of tissue tropism and host-range specificity (Holland et al., 1959; Holland, 1961). PVR is an integral membrane protein of the immunoglobulin (Ig) superfamily (Mendelsohn et al., 1989). PVR proteins, PVRx and PVRδ, which are alternative splicing isoforms, consist of a signal peptide, three Ig-like domains, a transmembrane domain and either one of the two cytoplasmic tails (Koike et al., 1990). Rodents lack the PVR molecule and are generally nonpermissive to PV infection. Mouse cells in culture and transgenic (tg) mice expressing PVR acquire PV susceptibility, indicating that PVR is a determinant of host-range specificity in mice (Mendelsohn et al., 1989; Ren et al., 1990; Koike et al., 1991). With regard to the tissue tropism, PV also plays an important role. In the tg mice, PVR mRNA expression was detected in the neurons in the CNS, and these neurons were infected by PV (Ren & Racaniello, 1992; Koike et al., 1994). Although PVR expression is necessary to acquire susceptibility to PV, it does not seem to be sufficient because PVR expression was observed in extraneural non-target tissues (Kunin & Jordan, 1961; Mendelsohn et al., 1989; Nomoto et al., 1994).

If the neuropathogenicity of PV is largely dependent on cell tropism, and if cell tropism is mainly determined by PVR expression in the CNS, PV would exhibit quite a different pathogenicity in tg mice expressing PVR in different patterns. In order to examine the influence of PVR expression on the neuropathogenicity of PV, we produced tg mice with different PVR distributions. Tg mice carrying the human genomic DNA for PVR (designated hg–PVR mice) have already been produced: ICR–PVRTg1 and ICR–PVRTg21 by our group (Koike et al., 1990). The hybridized DNA fragment of human genomic DNA for the PVR gene (designated as 'hg–PVR transgene'), as the 'hg–PVR mouse' for simplification in this paper.

**Methods**

- **Production of tg mice.** A full-length cDNA clone for the α form of PVR (Koike et al., 1990) was inserted into the EcoRI sites of the pCAGGS vector (Niwata et al., 1991). The plasmid, pCAG–PVR, was transfected into mouse L cells and the expression of the PVR protein on the cell surface was examined by immunofluorescent staining with the monoclonal antibody 4C6 (Aoki et al., 1994). A Sall–HindIII fragment of PVR–PVR, designated the ‘CAG–PVR transgene’, included the CAG promoter, PVR cDNA and the polyadenylation signal (Fig. 1A). This fragment was excised and purified by gel electrophoresis. Tg mice were produced by microinjection of the CAG–PVR DNA construct into the fertilized eggs of C57BL/6C3H10 mice as described previously (Koike et al., 1991). Tg mice were screened by PCR detection of the transgene using a set of primers specific for human PVR cDNA (PVR51: 5′-gtccagctcagtgaggacca-3′; PVR31: 5′-gctcaaatatt- agcctctgg-3′) from the DNA isolated from an ear biopsy specimen. PCR was performed in a 25 μl reaction mixture containing 0.4 μM of each primer, approximately 0.1 ng of template genomic DNA and 0.5 units of ExTag DNA polymerase (Takara) as recommended by the supplier. The amplification condition was 35 cycles each of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s. Three strains of tg mice, B6–CAG–PVRTg41, B6–CAG–PVRTg44 and B6–CAG–PVRTg55, were maintained in the hemizygous stage and used in this study. We refer to these strains as the ‘CAG–PVR mice’. ICR–PVRTg21 mice were maintained in the homozygous stage. They were maintained by sister–brother mating for more than 15 generations. The genetic background of ICR–PVRTg21 mice is therefore nearly uniform. We refer to ICR–PVRTg1 and ICR–PVRTg21 mice, which have a transgene of human genomic DNA for the PVR gene (designated as ‘hg–PVR transgene’), as the ‘hg–PVR mice’ for simplification in this paper.

- **Southern and Northern blot hybridizations.** Genomic DNAs were prepared from the liver of the mice. DNA was digested with restriction enzymes. Of the digests, 2 μg was separated by electrophoresis on a 1% agarose gel. Total RNA was isolated from the brain, spinal cord, heart, lung, liver, kidney, spleen and skeletal muscle of the tg mice by the acid guanidium thiocyanate–phenol–chloroform method (Chomczynski & Sacchi, 1987). Of the total RNA, 2 μg was glyoxylated and separated by agarose gel electrophoresis. Nucleic acids were transferred onto a nylon membrane, Hybond-N (Amersham) (Sambrook et al., 1989). A Sau–Sau cDNA fragment of human PVRx (nucleotide positions 98 to 856) labelled with 32P was used as a probe for Southern and Northern blot hybridizations. The copy number of the transgene was estimated by comparison with known amounts of plasmid DNA, pCAG–PVR. The filters were exposed to a Kodak X-OMAT AR film. The Northern blot was rehybridized with the G3PDH cDNA probe to confirm the intactness of RNA.

- **In situ hybridization.** An Xhol–Xhol human PVR cDNA fragment (nt 1146–1687) was inserted into the Sall site of pBluescript SK in both orientations. Plasmid DNA was digested with HindIII and transcribed in vitro using T7 RNA polymerase and the NTP mixture containing digoxigenin (DIG)–UTP (Roche) as substrates. Mice were fixed with 4% paraformaldehyde/PBS (PFA/PBS) by perfusion and overnight immersion at 4°C. Tissues were embedded in paraffin and sections at 7 μm thickness were prepared. In situ hybridization was carried out as described previously (Koike et al., 1994). The hybridized DIG-labelled RNA probe was detected and visualized using a DIG Nucleic Acid Detection kit (Roche). Sections were stained with methyl green to visualize the nucleic acids.

- **PV binding assay.** The mouse brain, spinal cord, heart, lung, liver, kidney, spleen and skeletal muscle were homogenized in 10 vols of ice-cold Dulbecco’s modified minimum essential medium (DMEM) using a
Polytron homogenizer. PV (1000 p.f.u.) was incubated with 1 ml of the tissue homogenates at 37 °C for 30 min. The virus titre of the virus–homogenate mixture was determined by plaque assay on an African green monkey kidney (AGMK) cell line, JVK-03 (Koike et al., 1992).

- PV infection in mice. The PV type 1 Mahoney strain was obtained by transfection of in vitro-transcribed RNA from an infectious cDNA clone, pOM (Shiroki et al., 1995). The RNA was isolated from the recovered virus and its sequence was verified. No nucleotide change was observed in the recovered virus compared with the sequence of the infectious cDNA clone. The virus was propagated in AGMK cells. The titre of stock virus was measured by plaque assay on the AGMK cells. Five-week-old mice were used in the infection experiments. The PV solution was inoculated into mice by intracerebral, intraperitoneal and intravenous routes (Koike et al., 1991). PV solution (25 µl) was also administered orally by dropping solution into the mouth.

- Measurement of neutralizing antibody titre. Two weeks after inoculation, the antibody titre of the serum of the surviving mice was measured by a microneutralizing test (World Health Organization, 1997). We considered that the mice elicited neutralizing antibody when virus was neutralized with the serum diluted more than fourfold.

- Immunohistochemistry. Mice that died following the virus infection were fixed in PFA/PBS. Mice were sacrificed by deeply anaesthetizing with ether and then perfused with PBS followed by PFA/PBS. The brains and the spinal cords were embedded in paraffin and 3 µm thick sections were prepared. PV antigens were detected with a rabbit anti-PV polyclonal antibody using an immunoperoxidase method (Koike et al., 1991).

Results

Establishment of tg mice

We generated tg mice that expressed human PVR under the transcriptional control of the CAG promoter. The CAG promoter consists of the chicken β-actin promoter and the enhancer of the cytomegalovirus immediate-early promoter, which is expected to allow ubiquitous expression in all cell types (Niwa et al., 1991). There are four splicing isoforms of PVR mRNA. Two of their products, PVRα and PVRβ, are transmembrane isoforms that function as a receptor. The efficiency of mediating PV infection is not different between the two isoforms in in vitro culture cells. We decided to introduce PVRα cDNA as the transgene. Nine strains of tg mice were identified by PCR out of 60 mice born from the infected eggs. All of these founders grew normally and eight of these were able to produce progeny. The transmission rates of the transgene in each strain were approximately 50% (data not shown). We decided to use three tg strains, B6–CAG–PVRTg41, B6–CAG–PVRTg44 and B6–CAG–PVRTg55, in this study.

Southern blot hybridization was carried out to confirm the presence and the integrity of the transgene (Fig. 1B). Bands with the expected size were found after restriction map analysis of the three strains, while no band was detected in DNA digest from non-tg mice. The copy numbers of the transgene in the three strains were roughly estimated to be between two and four.

PVR expression in tg mice

We determined PVR expression by four different methods. First, RT–PCR was carried out on total RNA isolated from various tissues of the mice using PVR51 and PVR31 as primers. A human PVR-specific band was observed in all tissues of tg mice tested but not in non-tg mice (data not shown). The specific band was observed only in the presence of reverse transcriptase in the reaction. Secondly, we performed Northern blot hybridization to investigate the expression of human PVR mRNA. The PVR transcript (approximately 2000 nt) was detected in all tissues of tg mice tested, but the expression level varied both among tissues and among tg strains (Fig. 1C). The same amount of RNA from non-tg mice did not contain any human PVR transcript (data not shown). The expression level
Neutralization of PV using tissue homogenates of mice. PV (10³ p.f.u.) was incubated with tissue homogenates. The PV titre after incubation was determined by plaque assay on AGMK cells. Data are represented as logarithmic values.

of the PVR mRNA was less than 10% of the endogenous β-actin mRNA in three tg strains. Thirdly, we tested the neutralization activity of PV using tissue homogenates of mice. The functional PVR protein is able to bind PV and it induces conformational changes of the virion, which initiates its uncoating. The neutralization activity of the tissue homogenate therefore indicates the presence of functional PVR protein. As shown in Fig. 2, all tissue homogenates had virus titres reduced to less than 10% of the levels of the inoculated virus, while no reduction in the virus titre was observed when tissue homogenates from non-tg mice were used. Fourthly, in situ hybridization was performed to determine the expression pattern of PVR mRNA in each cell type. In sections of the brain and spinal cord, signals from the PVR transcript were detected in the cytoplasm of almost all neurons, such as those in the cerebral cortex (data not shown), hippocampus (Fig. 3E), cerebellum (data not shown) and spinal cord (Fig. 3I). The PVR transcript was also detected in some glial cells (Fig. 3C, I) and ependymal cells (Fig. 3G). The hybridization signals from some glial cells were evident but some were not. No signal was observed in controls using sense PVR RNA as the probe in these cells (Fig. 3D, F, H, J, L). It is clear that PVR is expressed not only in neurons but also in ependymal cells and at least in some glial cells in CAG–PVR mice, although we could not conclude that the transgene expression is ubiquitous. The results were the same for the three CAG–PVR mouse strains.

PV infection in the tg mice

The PV type 1 virulent Mahoney strain was inoculated into tg mice by various routes. The hg–PVR mice showed flaccid paralysis and died 3–14 days post-inoculation (p.i.) by intracerebral, intraperitoneal and intravenous routes (Table 1) (Koike et al., 1991, 1994; Horie et al., 1994). These are essentially the same phenomena that are observed in PV-infected monkeys. By intracerebral route, the 50% lethal doses (LD₅₀) of the Mahoney strain in the ICR–PVRTg1 and ICR–PVRTg21 strains were 10¹⁵ and 10²⁴ p.f.u., respectively. More than 10⁹ p.f.u. of the PV type 1 Mahoney strain induced 100% mortality in hg–PVR mice (Table 1). Paralysis was not observed and neutralizing antibody was not detected in the serum of mice administered 10⁶ p.f.u. of PV orally (Koike et al., 1991).

In contrast, almost all CAG–PVR mice survived following PV infection by any route. No mice died after inoculation by intraperitoneal, intravenous and oral routes. Only a few CAG–PVR mice died after intracerebral inoculation of PV. Death was observed within at least 5 days p.i., mostly on day 1 or 2 p.i., when the virus titre in the brain reached the maximum level (Fig. 4). No mice died after 6 days p.i. Most of the inoculated mice of the three tg strains survived until the end of the observation period. The movements of infected mice were somewhat slow. The grip of the limbs of some mice were slightly weakened. However, apparent paralysis of the limbs was seldom observed.

Although CAG–PVR mice were not paralysed, we detected neutralizing antibodies against PV in the serum of surviving mice inoculated by intraperitoneal and intravenous routes (Table 1). The results suggest that the mice are susceptible to PV infection, but the virus did not reach the CNS following inoculation by peripheral routes, and/or extensive virus replication did not occur in the CNS, even though the virus reached the CNS. The result also suggests that the latter event does occur when virus is inoculated intracerebrally. We decided to analyse further the clinical and pathological changes following intracerebral inoculation.

Different mortality rates between the two tg strains are due to the difference in transgene expression

There are at least three possibilities to explain the difference in mortality rates between the two tg strains. It may be due to
Fig. 3. PVR expression in neurons and glial cells in CAG–PVR mice. In situ hybridization was carried out using an anti-sense RNA probe. A sense probe was used as a negative control. (A) Kruver–Barrera’s staining of coronal section of brain. Boxes indicate the region analysed for PVR mRNA expression by in situ hybridization in C–F. Original magnification, 6 × . (B) Kruver–Barrera’s staining of transverse section of spinal cord. Boxes indicate the region analysed for PVR mRNA expression by in situ hybridization in G–L. Original magnification, 6 × . (C) Corpus callosum region of the brain of B6–CAG–PVRTg55. Hybridization signals (purple precipitates) were observed in the cytoplasm around the nuclei (stained with methyl green) of the glial cells. Many glial cells expressed PVR mRNA, indicated by arrows. Original magnification, 120 × . (D) Negative control for (C). (E) Hippocampus region of B6–CAG–PVRTg55. PVR mRNA was detected in hippocampal neurons. Original magnification, 120 × . (F) Negative control for (E). (G) Spinal cord section of B6–CAG–PVRTg55 near the central canal. PVR mRNA was detected in ependymal cells indicated by the arrow. Methyl green staining was omitted. (H) Negative control for (G). (I) Spinal cord section of B6–CAG–PVRTg55 in the ventral horn region. PVR mRNA was detected in the motor neurons (arrowhead) and some glial cells (arrows). Original magnification, 120 × . (J) Negative control for (I). (K) Spinal cord section of B6–CAG–PVRTg55 in the ventral tunicii region. PVR mRNA was detected in most of the glial cells. Original magnification, 120 × . (L) Negative control for (K). Similar results were obtained using B6–CAG–PVRTg41 and B6–CAG–PVRTg44 mice.
Table 1. Mortality rate of tg mice after inoculation of PV

<table>
<thead>
<tr>
<th>Tg mice</th>
<th>PV inoculated (log₁₀ p.f.u.)…</th>
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<th>Intravenous</th>
<th>Oral</th>
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<td></td>
<td></td>
<td>6-4</td>
<td>6-4</td>
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</tr>
<tr>
<td>B6–CAG–PVRTg41</td>
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<td></td>
<td></td>
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<tr>
<td>B6–CAG–PVRTg55</td>
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<td>0(2)/3</td>
<td>0(0)/5</td>
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<tr>
<td>ICR–PVRTg21</td>
<td>4/4</td>
<td>4/4</td>
<td>0(0)/7</td>
<td></td>
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</table>

*B Number of dead/number of inoculated mice. The number of surviving mice that elicited neutralizing antibodies are indicated in parentheses.
† These results have previously been presented in Koike et al. (1994).

Fig. 4. PV recovered from the CNS of tg mice. Mice were inoculated with 10⁵ p.f.u. of poliovirus intracerebrally. Two or three mice were sacrificed daily and the brain and spinal cord were isolated. The tissues were homogenized in 5 ml DMEM and centrifuged at 3000 r.p.m. for 20 min at 4 °C. The virus titre of the supernatants was determined by plaque assay on AGMK cells. △, spinal cord of B6–CAG–PVRTg41 mice; ▲, brain of B6–CAG–PVRTg41 mice; □, spinal cord of ICR-PVRTg21 mice; ■, brain of ICR–PVRTg21 mice; ○, spinal cord of normal C57BL/6 mice; ●, brain of normal B6 mice.

Time courses of PV levels in the CNS of tg mice

We next recovered PV from the infected mice to examine whether the virus replicated in the CNS of the ICR–PVRTg21 and B6–CAG–PVRTg41 mice. The time courses of recovered virus in the two tg strains were quite different. In hgp–PVR mice, the amount of recovered virus increased and reached maximum level in the spinal cord on day 3 p.i. when the expression of PVRα both in neuronal and non-neuronal cells in the CAG–PVR mice, the difference in genetic background among strains, or the lack of PVRβ, PVRγ and PVRδ isoforms in CAG–PVR mice. To examine these possibilities, we mated homozygotes of ICR–PVRTg21 with hemizygotes of B6–CAG–PVRTg41. All of their F1 progenies have the same genetic background because both strains are inbred. Half of these had both the hgp–PVR and the CAG–PVR transgenes (hgp–PVR+/−, CAG–PVR+/−). The other half carried only the hgp–PVR transgene (hgp–PVR+/−, CAG–PVR−/−). All of the splicing isoforms of PVR are supposed to be expressed in neurons since all the mice carry the hgp–PVR transgene. PVRα is additionally expressed in many cells in the former tg littermates (hgp–PVR+/−, CAG–PVR+/−), but only in the neurons in the latter (hgp–PVR+/−, CAG–PVR−/−). Both genotypes of littermates were challenged by intracerebral inoculation of PV. The former were resistant to 10⁶ p.f.u. of PV (no mice died in 23 inoculated mice). All of the latter (20 inoculated mice) died at the same dose of virus. We therefore concluded that the difference in mortality rate is due to the PVR expression in non-neuronal cells in CAG–PVR mice, but not due to the difference in genetic background nor to the lack of different PVR isoforms.
Fig. 5. Histopathological changes in tg mice. (A) Krüver–Barrera’s staining of coronal section of brain. Boxes indicate the regions analysed for PV antigens in (C)–(E). Original magnification, 6×. (B) PV antigens of brain of B6–CAG–PVRTg44 mice. The mice died 2 days p.i. using the intracerebral route. Original magnification, 6×. (C) Detection of PV antigens in the ependymal cell layer around the lateral ventricles. Note that the lateral ventricle is narrowed. Original magnification, 60×. (D) and (E) Detection of PV antigens in the pyramidal neurons (Py) in the hippocampus (indicated by the arrow) and in the glial cells along with the corpus callosum (cc). Original magnification, 60×. (F) Krüver–Barrera’s staining of transverse section of the spinal cord. Boxes indicate the regions analysed for PV antigens in (H) and (I). Original magnification, 6×. (G) PV antigens in the spinal cord of B6–CAG–PVRTg55 mice. The mice died 2 days p.i. using the intracerebral route. (H) Detection of PV antigens in the glial cells in the dorsal funiculi. Original magnification, 120×. (I) Detection of PV antigens in motor neurons (arrowheads) and glial cells (arrow) in the ventral horn near the central canal. Original magnification, 120×. Similar results were obtained using all three CAG–PVR mouse strains.
most of the inoculated tg mice were paralysed and died. This indicated that the virus had spread from the inoculation site as a result of multiple cycles of infection.

In CAG–PVR mice, 10^6 p.f.u. of the virus was recovered from the brain on day 1 p.i. It is clear that PV multiplication occurred in the brain of CAG–PVR mice since the amount of virus recovered was greater than that of inoculated virus. After 2 days p.i., the virus titre began to decrease in the brain. This suggests that the virus multiplication may occur only in an early period and not spread efficiently from the initial multiplication site, or that virus clearance may begin as early as day 2 p.i. In the spinal cord, only a small amount of virus was recovered on day 2 p.i. The inoculated virus did not appear to spread to the spinal cord. These results explain the difference in the clinical symptoms and mortality rates between the two tg mouse strains.

Pathological changes in tg mice

The clinical symptoms and mortality rates greatly differed between hg–PVR and CAG–PVR mice after intracerebral inoculation of PV. We therefore examined the sites of virus multiplication and pathological changes of these mice. We have previously described the histopathological changes in hg–PVR mice (Koike et al., 1991; Horie et al., 1994). PV infection in the hg–PVR mice is restricted to neurons. This is in good agreement with the detection of PVR expression only in the neurons (Koike et al., 1994). Neurons in the ventral horn of the spinal cord, medulla oblongata, pons, thalamus and the midbrain were the major targets of PV. Infection of hippocampal neurons was sometimes observed but at a low frequency. Compared with the natural PV infection in humans and experimental infection in monkeys, cellular infiltration was minimal in hg–PVR mice, particularly in the case of intracerebral inoculation of virulent strains.

In contrast, major sites of PV replication in CAG–PVR mice were quite different from those in hg–PVR mice. In the brains of mice that died within 3 days p.i., ependymal cells degenerated with granulocyte infiltration. PV antigens were detected in the ependymal cells (Fig. 5B, C). In the corpus callosum and in the white matter between the lateral ventricle and the cerebral cortex, where no neurons exist, PV antigens were detected in glial cells (Fig. 5B, E). A preliminary experiment of double-labelling with PV antigen and an oligodendrocyte marker (glutathione S-transferase) suggested that the infected glial cells were mostly oligodendrocytes. PV antigens were also detected in pyramidal neurons in the hippocampus (Fig. 5B, D, E). PV antigens were not clearly detected in vascular endothelial cells and meningeal cells. All of the infected cells were localized within and around the ventricles, suggesting that the virus spread from this area. Neurons in the brainstem, which are often infected by PV in humans, monkeys and the hg–PVR mice (Bodian, 1955; Koike et al., 1991; Horie et al., 1994), were seldom infected. The lateral ventricle became narrowed or almost closed in all of the mice that died within 3 days p.i. (Fig. 5C). In the spinal cord, PV antigens were detected in the motor neurons in the ventral horn, ependymal cells, neurons and glial cells near the central canal, and glial cells in the white matter of dorsal funiculi (Fig. 5G, H, I). These results indicate that many cell types, including neurons, glial cells and ependymal cells, became susceptible to PV when they expressed PVR. The ependymal cells seem to be...
positive cells were detected. brain and spinal cord (Fig. 4). On day 14 p.i., few PV-antigen-positive cells was consistent with the results of virus recovery from the brain and spinal cord. The time course of the number of PV-antigen-positive cells was maximum on day 2 p.i. and gradually decreased. Only a very few PV-antigen-positive cells were detected in the spinal cord on day 2 p.i. and there were none detected in all tissues examined. The expression levels of the transgene varied from strain to strain. However, the clinical and pathological changes caused by PV infection were essentially the same among CAG–PVR tg strains. A strict tropism towards neurons was absent in the CAG–PVR mice, suggesting that the glial and ependymal cells are potentially susceptible to PV infection provided that PVR is expressed. This experimental evidence suggests that the glial and ependymal cells are potentially susceptible to PV infection that PVR is expressed. The expression levels of the transgene varied from strain to strain. However, the clinical and pathological changes caused by PV infection were essentially the same among CAG–PVR tg strains. A strict tropism towards neurons was absent in the CAG–PVR mice, suggesting that the glial and ependymal cells are potentially susceptible to PV infection provided that PVR is expressed. This experimental evidence suggests that the glial and ependymal cells are potentially susceptible to PV infection.

**Ventricular dilation caused by PV infection**

Most of the CAG–PVR mice survived until the end of the observation period. We observed for as long as 8 weeks p.i. We examined the time-course of pathological changes. B6–CAG–PVRTg41 mice were inoculated with $10^{6.7}$ p.f.u. of PV and sacrificed on days 2, 3, 5, 14, 28, 42 and 56 p.i. On day 14 p.i., ventricular dilation became evident (Fig. 6). Mild ventricular dilation without cortical thinning was observed in mice in the control experiments, using non-tg mice inoculated with PV and tg mice inoculated only with DMEM without virus. The ventricular dilation observed in tg mice inoculated with PV is greater and is associated with cortical thinning. Table 2 shows the incidence of severe ventricular dilation associated with cortical thinning on day 14 p.i. Ventricular dilation occurred more frequently as the inoculated virus titre increased. Dose dependency was most clearly seen in B6–CAG–PVRTg55 mice. Dilation proceeded in a time-dependent manner in B6–CAG–PVRTg41 mice (Fig. 6).

**Table 2. Incidence of ventricular dilation in CAG–PVR mice after intracerebral inoculation of PV**

<table>
<thead>
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<th>PV inoculated (log10 p.f.u.)</th>
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<td>C57BL/6(non-tg)</td>
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hydrocephalus has also been observed in hamsters experimentally infected with mumps virus (Johnson et al., 1967). Events similar to those of mumps virus infection in rodents may occur in PV infection in CAG–PVR mice. PVR might be expressed in cells that are responsible for causing ventricular dilation. Destruction of ependymal cells or oligodendrocytes in the corpus callosum may be a mechanism for causing such pathological change.

**Discussion**

To determine how the PVR distribution affects the cell tropism and pathogenesis of PV, we compared the clinical and histopathological changes in two different genetically engineered mice. In the CAG–PVR mice, PVR expression was detected in all tissues examined. The expression levels of the transgene varied from strain to strain. However, the clinical and pathological changes caused by PV infection were essentially the same among CAG–PVR tg strains. A strict tropism towards neurons was absent in the CAG–PVR mice, suggesting that the glial and ependymal cells are potentially susceptible to PV infection that PVR is expressed. This experimental evidence suggests that the PVR protein is expressed in neurons but not in glial cells in humans.

There is an example in which a change of cell tropism alters the pathogenicity of a virus. Neuroviral strains of mouse hepatitis virus (MHV) produce an acute and often fatal encephalitis with extensive neuronal involvement, whereas neuroattenuated variant strains spread only slowly in neurons and generally infect glia, with attendant chronic white matter disease (Fleming et al., 1986). These pathological changes induced by MHV strains are similar to those in hg–PVR mice and those in CAG–PVR mice induced by PV, respectively.
This observation is consistent with the idea that the acquisition of susceptibility in the non-neuronal cells alters the virus phenotype into an attenuated one.

Among members of the *Picornaviridae*, CVs and echoroviruses invade the CNS, cause aseptic meningitis and terminate with good recovery in many cases (Johnson, 1998). The clinical and histopathological changes in CAG–PVR mice after PV infection were mainly on the ependymal surface. The parenchyma of the brain and the spinal cord were not severely damaged in most cases. This resembles the pathological changes observed in a patient (Price et al., 1970) who died of myocarditis accompanied by meningitis due to CVB5 infection, rather than those observed in PV infection in human and hg–PVR mice. The cellular receptor for CVBs, the coxsackievirus–adenovirus receptor (CAR), has been identified (Bergelson et al., 1997, 1998; Tomko et al., 1997). The distribution of CAR in the human CNS is not known. However, CAR is expressed in ependymal cells in rats (Johansson et al., 1999). It is possible that CAR is expressed in the ependymal and glial cells in the human CNS and that CVBs do not frequently cause fatal encephalitis because of these non-neuronal cells.

The most interesting observation is that the viral invasion of the CNS did not spread and did not result in the death or paralysis of infected CAG–PVR mice, although the neurons are susceptible to the virus, similar to those of hg–PVR mice. This was most evident in the motor neurons in the spinal cord of the CAG–PVR mice. A small amount of virus was recovered and a few antigen-positive cells were detected in the spinal cord of CAG–PVR mice on day 2 p.i., but infection did not spread to larger areas to cause paralysis. Virus clearance may have occurred as a result of adsorption and neutralization of infectious particles by PVR protein ectopically expressed. This prevents secondary infection near the initial infection sites. Alternatively, immunological responses may have begun from the early stage of infection only in CAG–PVR mice. Inflammatory cells are observed as early as day 2 p.i. in CAG–PVR mice, whereas cell infiltration was not observed at such an early period of infection in hg–PVR mice. This suggests that the immune response was induced to clear the virus in the CAG–PVR mice before the virus infected neurons that are essential for the maintenance of life. Non-neuronal cells may have a much greater capacity to induce immunological response than neurons in the CNS. The CNS shows a generalized lack of constitutive major histocompatibility complex (MHC) expression. Microglia, oligodendrocytes and endothelial cells, but not neurons, were induced to express MHC molecules in the CNS infected with viruses or treated with interferon–γ (Joli et al., 1991; Horwitz et al., 1999). In the CAG–PVR mice, infected non-neuronal cells would likely be MHC class I-positive and therefore could be a good target for an anti-viral response. In contrast, neurons would not express sufficient levels of the MHC class I molecule in hg–PVR mice. The infecting PV is therefore able to escape immunological surveillance. In our preliminary experiments, the CAG–PVR mice became resistant to infection by Japanese encephalitis virus (JEV), when JEV was inoculated intracerebrally 1 day after PV inoculation (data not shown). This suggested that an anti-virus state was established by PV infection in CAG–PVR mice. We are now investigating immune responses in CAG–PVR mice.

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References


Poliovirus pathogenicity in transgenic mice


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