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Human Poliovirus Receptor Gene Expression and Poliovirus Tissue Tropism in Transgenic Mice

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Expression of the human poliovirus receptor (PVR) in transgenic mice results in susceptibility to poliovirus infection. In the primate host, poliovirus infection is characterized by restricted tissue tropism. To determine the pattern of poliovirus tissue tropism in PVR transgenic mice, PVR gene expression and susceptibility to poliovirus infection were examined by in situ hybridization. PVR RNA is expressed in transgenic mice at high levels in neurons of the central and peripheral nervous system, developing T lymphocytes in the thymus, epithelial cells of Bowman's capsule and tubules in the kidney, alveolar cells in the lung, and endocrine cells in the adrenal cortex, and it is expressed at low levels in intestine, spleen, and skeletal muscle. After infection, poliovirus replication was detected only in neurons of the brain and spinal cord and in skeletal muscle. These results demonstrated that poliovirus tissue tropism is not governed solely by expression of the PVR gene nor by accessibility of cells to virus. Although transgenic mouse kidney tissue expressed poliovirus binding sites and was not a site of poliovirus replication, when cultivated in vitro, kidney cells developed susceptibility to infection. Identification of the changes in cultured kidney cells that permit poliovirus infection may provide information on the mechanism of poliovirus tissue tropism.

Viral infections are often localized to specific cells and tissues within the host. This cell and tissue tropism results in distinct disease patterns for different viruses. Because all viruses initiate infection by binding to a specific receptor on the cell surface, the virus-receptor interaction has long been considered the first determinant of tissue tropism. For some viruses, such as human immunodeficiency virus type 1 (18) and Epstein-Barr virus (1), expression of cell receptors appears to control the pattern of virus infection in the host. However, viral replication in the host may be blocked at steps in the viral life cycle other than receptor binding. For example, the receptor for influenza virus, sialic acid, is ubiquitous, yet viral replication is largely limited to the respiratory tract. Influenza virus tropism is most likely determined by the availability of cellular proteinases required for cleavage of the hemagglutinin (8; reviewed in reference 27).

Poliovirus, the causative agent of paralytic poliomyelitis, is a human enteric virus that is classified into three serotypes. Most polioviruses have a distinct species tropism and only infect primates (reviewed in reference 21). In the primate host, poliovirus infection is characterized by a restricted tissue tropism; poliovirus replicates only in the pharynx and gut and in neurons within certain regions of the central nervous system (CNS), despite the presence of virus in many organs during the viremic phase of infection (3, 26). The molecular basis for this restricted tropism is not known. Assays for virus binding activity in tissue homogenates revealed a correlation between poliovirus binding and susceptibility to poliovirus infection (9). However, virus has also been reported to bind homogenates of tissues that are not sites of poliovirus replication (9, 17).

A human cell receptor for poliovirus (PVR) has recently been identified as a novel member of the immunoglobulin superfamily of proteins (19). PVR RNA and protein are expressed in a wide range of human tissues, including those that are not sites of poliovirus infection (7, 19). However, little is known about PVR gene expression in individual cell types. It is possible that cells expressing PVR within nonsusceptible tissues are not accessible to poliovirus, or perhaps only a small fraction of cells in these tissues express PVR and as a result virus growth is not detected. Restriction of PVR expression to neurons within the brain and spinal cord might explain the selective vulnerability of these cells to poliovirus infection. Alternatively, poliovirus tissue tropism may not be governed solely by expression of PVR but may depend on tissue- or cell-specific modification of the PVR, additional factors required for PVR function, or perhaps factors required for subsequent stages in virus replication.

We previously established transgenic mice containing the human PVR gene in the germ line (PVR transgenic mice) (24). The transgenic mice express PVR transcripts and poliovirus binding sites in a wide range of tissues. Inoculation of PVR transgenic mice with poliovirus leads to development of a fatal paralytic disease that clinically and histopathologically resembles human poliomyelitis. Absence of PVR is therefore the determinant of poliovirus host range restriction in mice. To characterize the tropism of poliovirus infection in PVR transgenic mice, the location of poliovirus replication in infected animals was determined by in situ hybridization and compared with the distribution of PVR transcripts in different cell types. Within the brain and spinal cord, PVR transcripts and poliovirus replication were detected only in neurons. However, despite the expression of PVR transcripts in specific cell types of many nonneural tissues, the only other site of poliovirus replication detected was skeletal muscle. Transgenic mouse kidney, which expresses PVR transcripts and virus binding sites but is not susceptible to poliovirus infection, developed permissivity to infection after cultivation in vitro, providing a model for studying the mechanism of resistance to poliovirus infection.

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MATERIALS AND METHODS

Hybridization probe synthesis. A 1,263-bp *Sma*I-*Xho*I fragment of PVR cDNA H20B, which contains most of the PVR coding sequences, was subcloned into plasmid vector pBluescript KS(−) and designated pBS5R. For in situ hybridization, antisense probes labeled with [^{35}S]UTP to a specific activity of 2×10^6 to 4×10^6 cpm/ng were generated by using *Bam*HI-digested pBS5R as template for transcription by T3 RNA polymerase. Alternatively, the control sense strand was synthesized with *Xho*I-digested pBS5R template and T7 polymerase. Similarly, the 1,452-bp *Bgl*II-*Pvu*II fragment of poliovirus type 1 Mahoney (P1/Mahoney) cDNA, containing poliovirus polymerase coding sequences, was subcloned into plasmid vector pBluescript KS(−) and designated pBSMP. Antisense probe was generated by using *Sac*I-digested pBSMP and T3 polymerase, and sense probe was synthesized from *Cla*I-digested pBSMP by T7 polymerase. The RNA was hydrolyzed at pH 10.2 at 60°C, as described previously (11), to yield probes approximately 100 bp in length. Probe length before and after hydrolysis was assessed on formaldehyde-agarose gels.

In situ hybridization. Mice were sacrificed, and the brain, spinal cord, thymus, lung, intestine, spleen, kidney, adrenal gland, and hindlimb muscle were removed and fixed by overnight immersion in 4% paraformaldehyde at 4°C. The paraformaldehyde was replaced with phosphate-buffered saline (PBS), and tissues were washed, dehydrated, and embedded in paraffin wax as described previously (11). Embedded tissues were sectioned at 6 μm and collected on Tespa-treated slides. Hybridization, exposure to photoemulsion (3 to 6 days for PVR probes and 1 day for poliovirus probes), counterstaining, and microphotography were as described previously (11). Positive hybridization was determined by comparison with background levels, defined by using sense probes on transgenic mouse sections and antisense probes on nontransgenic mouse sections.

Cells, virus, and mice. Mice were sacrificed, and the kidneys were removed, washed three times with Dulbecco's modified Eagle's medium (DMEM) (GIBCO), and sliced into small pieces. The tissue slices were then treated with 10 mg of collagenase H (Boehringer Mannheim Biochemicals) per ml that had been dissolved in DMEM and sterile filtered for 3 h at 37°C. The suspension was centrifuged at room temperature at $1,000 \times g$ for 10 min. The cell pellet was resuspended in 10 ml of enzyme-free cell dissociation solution (Specialty Media, Inc.), incubated at 37°C for 15 min and centrifuged at $1,000 \times g$ for 10 min. The cell pellet was either resuspended in DMEM containing 10% fetal bovine serum, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 10 μg of gentamicin per ml for culture in monolayers or cultured in suspension as described previously (23). For culture in monolayer, the cells were placed in 25-cm^2 tissue culture flasks pretreated with 0.5% gelatin at 37°C overnight.

HeLa S3 cells were grown in monolayer as described previously (23). P1/Mahoney strain was derived from the infectious cDNA clone (22). Three-week old mice of the PVR transgenic line TgPVR1-17 (24) were used for all experiments.

Virus growth and assay. Monolayers and suspension cultures of transgenic mouse kidney cells were infected with P1/Mahoney at a multiplicity of infection of 10. After 60 min of adsorption, the plates were washed three times to remove unattached viruses and the medium was replaced. Aliquots of the supernatant were removed at different times after infection, and virus titers were determined by plaque assay on HeLa cell monolayers. Tenfold serial dilutions of viruses, prepared in PBS plus 0.2% horse serum, were used to inoculate 6-cm dishes of HeLa cells and adsorbed at 37°C. The cells were then covered with 5 ml of 0.9% Bacto-Agar (Difco Laboratories) in DMEM plus 5% horse serum. After incubation at 37°C in 5% CO_2 for 2 to 3 days, plates were stained with crystal violet as described previously (23).

For virus binding assay, dispersed kidney cells from approximately 50 mg of fresh kidney were mixed with 10^6 PFU of P1/Mahoney and incubated at 37°C for 2 h. Cells were removed by centrifugation, and the remaining infectious virus in the supernatant was determined by plaque assay. Percent binding was calculated by subtracting the amount of virus remaining in the supernatant from the input virus, determined after mock incubation in PBS. Results are the average of two experiments.

PCR amplification of cDNA. Preparation of total cell or tissue RNA and oligo(dT)-primed cDNA synthesis was as described previously (19). Quantitative polymerase chain reaction (PCR) amplification of cDNA was carried out as described previously (29), using PVR-specific primers flanking the transmembrane domain (nucleotides 1170 to 1191 and 1422 to 1443; numbered as in reference 19).

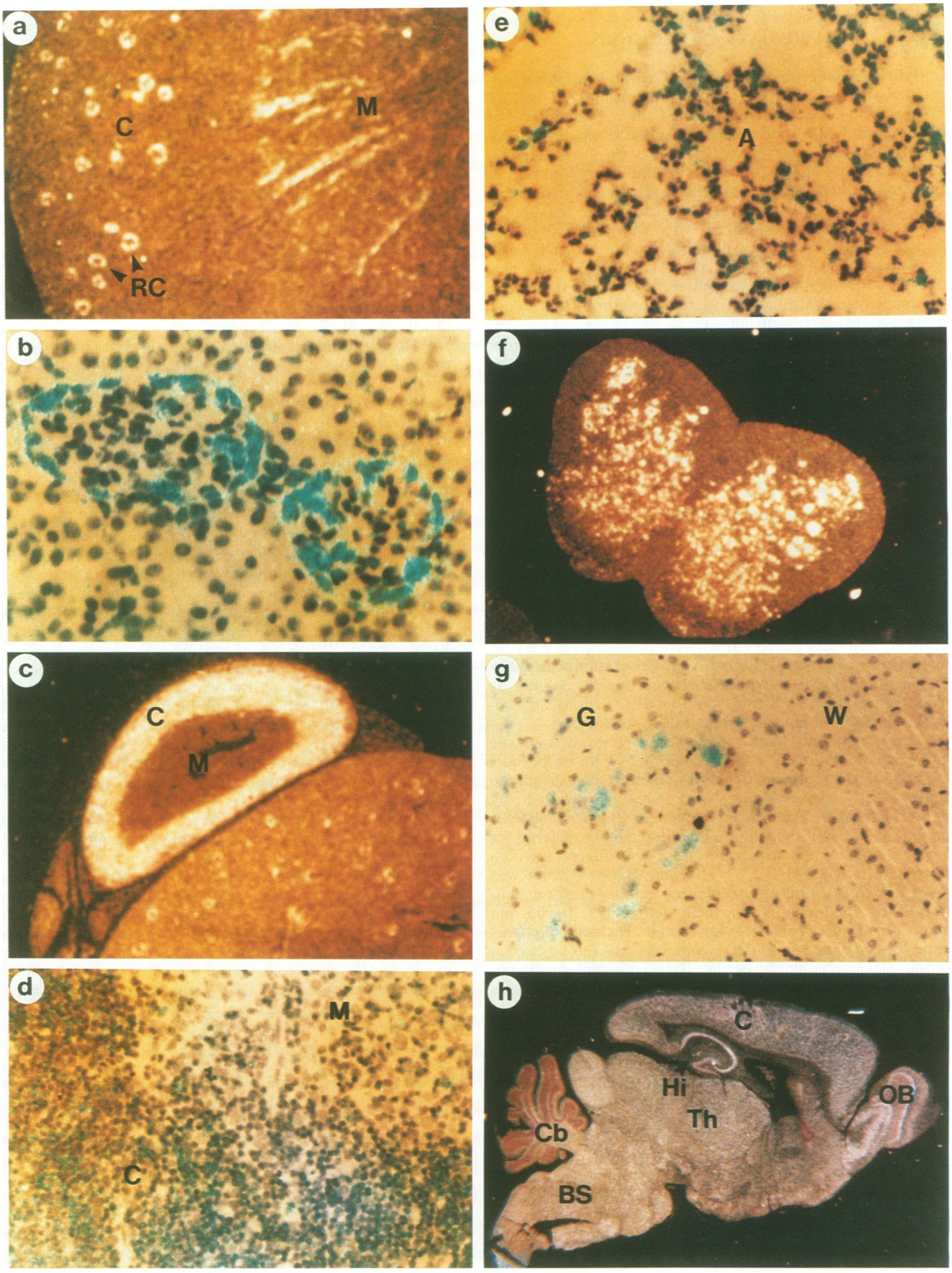
RESULTS

Localization of PVR RNA in transgenic mouse tissues. Previous analysis of PVR gene expression by Northern (RNA) blot hybridization showed that PVR transcripts are expressed at different levels in a wide range of transgenic mouse organs (24). High-stringency in situ hybridization with radiolabeled PVR RNA probes was used to examine the distribution of PVR RNA in specific cell types.

Expression of the PVR in transgenic mouse CNS was examined by hybridizing transverse sections of adult spinal cord and sagittal sections of the brain with antisense PVR RNA probes. Most neurons in all areas of the CNS expressed high levels of PVR transcripts (Fig. 1F to H). All neurons in the spinal cord, including motor and autonomic neurons and interneurons, expressed high levels of PVR RNA. In the brain, PVR RNA was detected in neurons of all cortical layers of the cerebral cortex and cerebellum and in nuclei of the brain stem, hippocampus, thalamus, hypothalamus, amygdala, pyriform cortex, basal ganglia, and olfactory bulb. The signals observed in spinal cord neurons were consistently higher than those observed in the brain. Within the brain, high levels of PVR transcripts were detected in neurons of the cerebral cortex and brain stem, in pyramidal cell layers of the hippocampus and pyriform cortex, and in amygdala, the mitral cell layer of the olfactory bulb, and the anterior olfactory nucleus (Fig. 1H). Neurons in the peripheral nervous system, such as parasympathetic ganglia, also showed high levels of expression (25).

In the kidney, high levels of PVR transcripts were detected in epithelial cells of the parietal layer of Bowman's capsule, possibly also in podocytes (the visceral layer of Bowman's capsule) in the glomerulus, and in some tubular epithelial cells in the medulla, predominantly in the outer stripe of the outer zone and the renal papilla (Fig. 1a and b). PVR transcripts were not detected in the renal pelvis, nor in lymph nodes, fatty tissue, or blood vessels that surround the kidney.

In the lung, PVR transcripts were detected in cells, tentatively identified as macrophages, lining the alveoli (Fig. 1e). Bronchial epithelial cells expressed lower levels of PVR transcripts. PVR RNAs were also detected in T lymphocytes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in the cortex of the thymus, as well as in some cells in the medulla of the thymus and in endocrine cells of the adrenal cortex (Fig. 1c and d). PVR gene expression was detected at low levels in most cells of the intestine, spleen, and skeletal muscle (25).

Alternatively spliced forms of PVR transcripts have been described that encode two soluble forms of PVR that lack the transmembrane domain (15). Transcripts encoding both membrane-bound and soluble PVR were detected in all organs tested of another line of PVR transgenic mice (16). Using PCR amplification of cDNA, PVR RNA encoding membrane-bound PVR and two soluble forms of PVR was detected in all organs examined in the PVR transgenic mice studied here. For example, all three forms were detected in brain and kidney, as well as in HeLa cells (Fig. 2). Nucleotide sequence analysis of PCR products indicated that the alternatively spliced forms are identical to those reported previously (15).

Poliovirus replication sites in the CNS of transgenic mice. To identify the sites of virus multiplication in the CNS, mice were infected intraperitoneally or intracerebrally and tissues of paralyzed animals were examined by in situ hybridization with viral RNA probes.

In paralyzed transgenic mice, spinal cord lesions were the most severe in the neuraxis, with inflammation and neuronal degeneration localized largely to the ventral horns. Infected neural cells were detected by in situ hybridization in all areas of the grey matter, including the ventral horn, intermediate and intermediolateral columns, and dorsal horn (Fig. 3). Viral RNA was detected in the cytoplasm of neurons, both in the cell bodies and in their axonal and dendritic processes. Diffuse hybridization was often observed around lysed neurons in areas containing inflammatory cells (Fig. 3a). Viral RNA was not detected in vascular endothelial cells or glial cells. Sites of lesions in the spinal cords of paralyzed animals corresponded with the distribution of clinical paralysis. For example, in mice with left leg paralysis, viral RNA was detected in most neurons on both sides of the lumbar spinal cord, but neuronal destruction was observed only in the left ventral horn of the cord. As infection progressed, other neurons within the same side of the cord were infected; at later stages, neuronal destruction was present on the affected side, and virus spread to both motor neurons and interneurons of the unaffected side and to upper segments of the spinal cord and brain stem.

In the brains of paralyzed mice that had been inoculated intraperitoneally, most sites of viral replication were in the brain stem, accompanied by marked microglial proliferation and perivascular cuffing. Viral replication was not detected in the cerebral cortex, cerebellum, hippocampus, thalamus, hypothalamus, or olfactory bulb. It was not clear whether

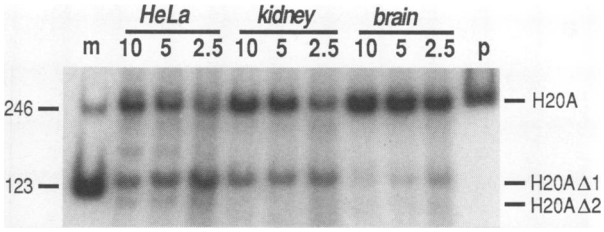


FIG. 2. Detection of alternatively spliced PVR RNA. PVR mRNAs were amplified by quantitative PCR of cDNA, using 5' ^{32}P-labeled primers flanking the PVR transmembrane region, and fractionated on a 6% polyacrylamide gel. Total RNA (10, 5, or 2.5 μg) was used for cDNA synthesis and/or amplification. Size markers (m) of 123 and 246 bp are indicated. Lane p, products of PCR, using PVR cDNA as template. The positions of amplified products from H20A, H20AΔ1, and H20AΔ2 are shown; nucleotide sequence analysis confirmed that these are derived from mRNAs encoding membrane-bound PVR and two soluble forms of PVR. H20AΔ2 products are visible in tissues after long exposure of the autoradiograph.

neurons in these regions were not susceptible to poliovirus or whether virus was unable to reach these areas after intraperitoneal inoculation. To address this question, transgenic mice were infected intracerebrally and brains from paralyzed mice were examined by in situ hybridization (Fig. 4). The results indicated that neurons in the brain stem were extensively infected, and viral replication was also detected in neurons in many other areas of the brain, including the cerebral cortex, pyramidal layer of the hippocampus, olfactory bulb, thalamus, hypothalamus, and deep cerebellar nuclei.

Poliovirus susceptibility of transgenic mouse nonneural tissues. PVR transgenic mice developed paralytic disease after intramuscular inoculation with P1/Mahoney (25). To determine whether poliovirus could replicate in muscle, mice were inoculated intramuscularly with 5×10^5 PFU of P1/Mahoney and, at different times after infection, the hamstrings of three mice were removed and homogenized, and virus titers were determined by plaque assay. Levels of virus in muscle rose to 10^6 PFU/mg by day 4 postinfection (Fig. 5a), at which time paralysis was observed. In contrast, poliovirus did not replicate in muscle cells of nontransgenic mice. Examination of infected PVR transgenic mouse muscle by in situ hybridization, using a viral RNA probe, revealed poliovirus replication in muscle cells (Fig. 5b).

Intravenous and intraperitoneal inoculation of PVR transgenic mice with poliovirus leads to development of poliomyelitis (24). To determine whether nonneural tissues which express PVR transcripts can support poliovirus infection,

FIG. 1. PVR mRNA expression in transgenic mouse tissues. Sections were hybridized with ^{35}S-labeled antisense PVR RNA probe. Silver grains, indicating positive hybridization, appear bright white in dark-field photomicrographs (a, c, f, and h) or bright green in polarized light epiluminescence photomicrographs (b, d, e, and g). (a) Kidney section showing cortex (C) and medulla (M). Strong hybridization signals localized specifically in renal corpuscles (RC) and some renal tubules. Magnification, ×106. (b) Higher magnification of renal corpuscles. High-level PVR mRNA expression in epithelial cells of the parietal layer of Bowman's capsule and possibly also in podocytes (the visceral layer of Bowman's capsule) in the glomerulus. Magnification, ×663. (c) Section of adrenal gland showing cortex (C) and medulla (M). Strong hybridization signals localized specifically in the adrenal cortex. Magnification, ×106. (d) Section of thymus showing cortex (C) and medulla (M). Strong hybridization signals in T lymphocytes in the cortex and some cells in the medulla. Magnification, ×663. (e) Section of lung showing alveoli (A). Some alveolar lining cells show strong hybridization signals. Magnification, ×663. (f) Transverse section of lumbar spinal cord. Strong hybridization signals in grey matter. Magnification, ×66. (g) Higher magnification of spinal cord transverse section; grey (G) and white (W) matter are labeled. High levels of PVR mRNA in all neurons. Magnification, ×265. (h) Sagittal section of brain. Regions of the brain that show PVR expression include the olfactory bulb (OB), cerebral cortex (C), hippocampus (Hi), thalamus (Th), brain stem (BS), and cerebellum (Cb). Magnification, ×16.

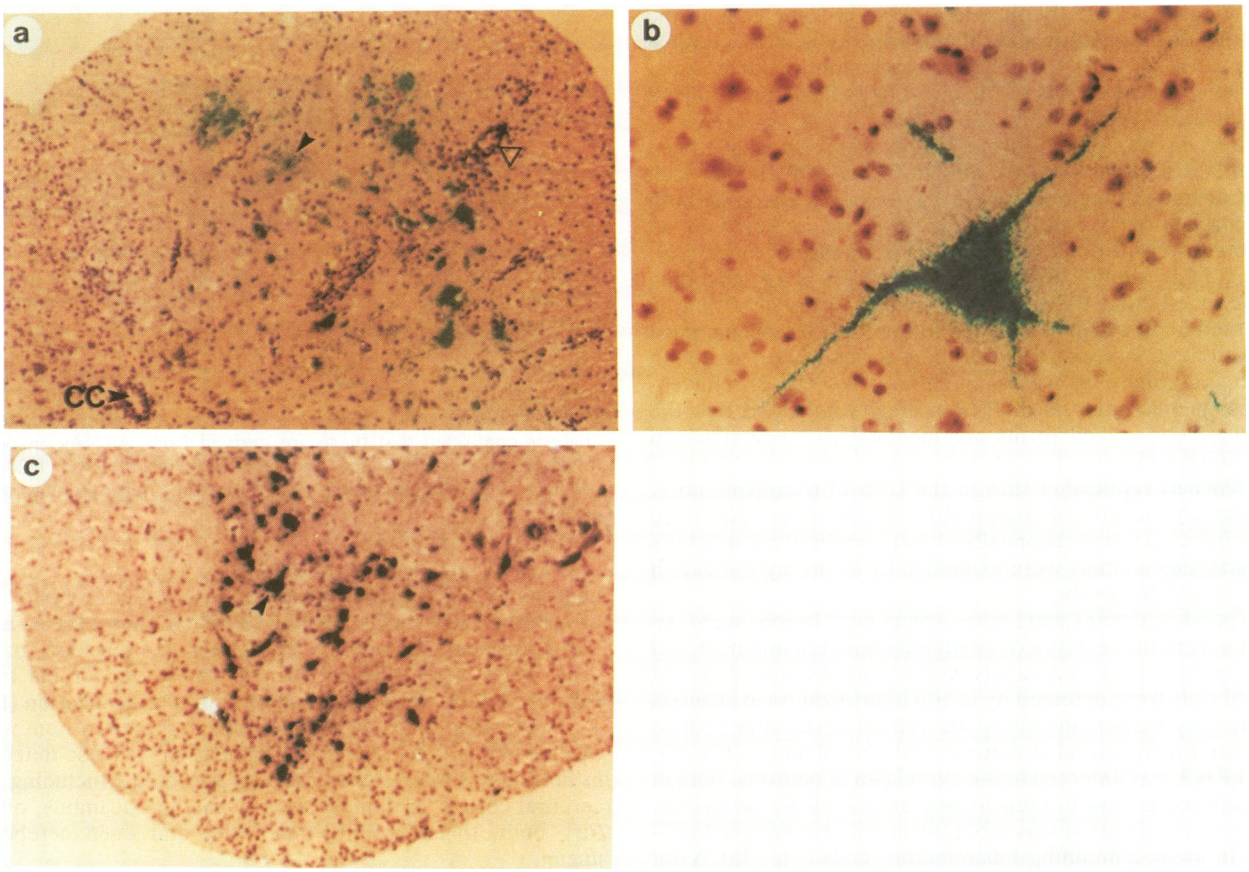


FIG. 3. In situ detection of poliovirus RNA in spinal cord of PVR transgenic mice infected intraperitoneally with poliovirus. Sections were hybridized with ^{35}S-labeled antisense P1/Mahoney viral RNA probe. Silver grains, indicating positive hybridization, appear bright green in the polarized light epiluminescence photomicrographs. (a) Lumbar spinal cord, left ventral horn. Strong hybridization signals are present in neurons. Some neurons have lysed (filled arrowhead). Severe inflammation with perivascular cuffing (open arrowhead) and diffuse and focal proliferation of microglial cells are seen. Hybridization is not detected in neuroglial cells in both the white and grey matter nor in the central canal (CC). Magnification, ×210. (b) Higher magnification of poliovirus-infected neuron in the ventral horn of the spinal cord. Poliovirus RNA is present both in the cell body and in axonal and dendritic processes. Magnification, ×843. (c) Lumbar spinal cord, left dorsal horn. Neurons were infected with poliovirus (arrowhead) in the absence of significant inflammatory changes. Magnification, ×210.

the ability of poliovirus to replicate in kidney and thymus was determined. PVR transgenic mice were inoculated intravenously with 10^6 PFU of P1/Mahoney, and at different times after infection organs were removed and homogenized, and virus titer was determined by plaque assay. Virus titer in both kidney and thymus declined within the first 3 days after infection, suggesting that virus replication did not occur at these sites (25). Poliovirus also failed to replicate in liver and spleen after intravenous inoculation and in the intestine after oral inoculation (25). However, it was possible that the inoculated virus did not have the opportunity to infect susceptible cells, because virus in the blood is quickly removed by the reticuloendothelial system (26). To examine this possibility, transgenic mice were inoculated intraperitoneally with 5×10^7 PFU of P1/Mahoney and virus levels in kidney were determined by plaque assay. Virus titers in both transgenic and nontransgenic kidney declined rapidly after inoculation, and no virus was detected in this organ after day 2 postinfection (Fig. 6). Paralysis was observed in many of these animals, indicating that virus had reached the CNS.

TABLE 1. Susceptibility of PVR transgenic mouse kidney cells after in vitro cultivation

Day postdispersion of PVR transgenic mouse kidney cells	Poliovirus susceptibility[a]	
	Monolayer	Suspension
0	ND	R
1	S	S
2	S	S
3	S	ND

[a] Determined by plaque assay in HeLa cell monolayers. R, resistant to infection: no increase in poliovirus titer observed 24 h postinfection; S, susceptible to infection: poliovirus titers increased 2 to 6 $\log_{10}$ PFU 24 h postinfection, with nearly complete destruction of cell monolayers; ND, not done. Cultured kidney cells from normal mice did not develop susceptibility to poliovirus infection. Collagenase treatment did not affect poliovirus susceptibility of HeLa cells or 24-h cultures of PVR transgenic mouse kidney cells.

Because only a small fraction of kidney cells express PVR, virus growth in these cells might not be detected by plaque assay of kidney homogenates. Therefore, poliovirus replication in transgenic mouse kidney and surrounding tissues was examined by in situ hybridization, using an antisense viral RNA probe. Viral replication was not detected in kidney, adrenal gland, connective tissue, fatty tissue, lymph node, parasympathetic ganglia, blood cells, or blood vessels for 1

week after intraperitoneal inoculation, although paralytic disease developed in some of these animals (25).

Susceptibility of cultured PVR transgenic mouse kidney cells to poliovirus. Although poliovirus tropism in primates is restricted, cells from almost any tissue become susceptible to poliovirus infection after cultivation in vitro (6, 9, 13). To examine the susceptibility of cultured PVR transgenic mouse kidney cells to poliovirus, kidneys were dispersed with collagenase and cultivated either in monolayers or in suspension and infected with poliovirus at a multiplicity of infection of 10. Although freshly isolated transgenic mouse kidney cells were resistant to poliovirus infection, after 24 h of growth in culture, the cells became highly susceptible to poliovirus infection (Table 1). Cultured kidney cells from normal mice did not develop susceptibility to poliovirus infection. Freshly dispersed, poliovirus-resistant transgenic mouse kidney cells expressed PVR at the cell surface, as determined by poliovirus binding assay. Transgenic kidney cells bound 90% of the input virus (10^6 PFU), while nontransgenic kidney cells failed to bind poliovirus. Despite expression of poliovirus binding sites on the cell surface, freshly dispersed PVR transgenic mouse kidney cells are resistant to poliovirus infection.

DISCUSSION

The factors that control the selective vulnerability of primate cells to poliovirus infection are not known. To understand the basis of poliovirus tropism, we have studied a transgenic mouse line expressing the human PVR. The pattern of poliovirus infection in transgenic mice was determined and compared with the expression of PVR transcripts. The results show that PVR RNA is expressed at a high level in neurons of both the central and peripheral nervous systems, in T lymphocytes in the thymus, in epithelial cells of Bowman's capsule and some tubules in the kidney, possibly in alveolar macrophages in the lung, and in endocrine cells in the adrenal cortex. High-level PVR RNA expression was not detected in intestine, spleen, and lymph nodes. Although PVR expression was widespread, when transgenic mice were inoculated with poliovirus, viral replication was limited to skeletal muscle and the CNS. Poliovirus RNA was detected in muscle cells of the hamstring after intramuscular inoculation. In addition, we have recently obtained evidence that poliovirus replicates in the muscles of transgenic mice after oral administration of virus (25). Replication of poliovirus has also been demonstrated in monkey skeletal muscle after intramuscular inoculation (28). Poliovirus RNA was detected in all neurons of the spinal cord and in most neurons in the brain stem. In the brain, infected neurons were detected in several areas, including the cerebral cortex, pyramidal layer of the hippocampus, olfactory bulb, thalamus, hypothalamus, and deep cerebellar nuclei. Poliovirus replication was not detected in kidney, adrenal gland, thymus, and intestine. Susceptibility of cells to poliovirus therefore appears to correlate with PVR RNA expression in the CNS and in muscle, but not in other tissues.

There are several possible explanations for the failure of poliovirus to replicate in transgenic mouse tissues that express the PVR. It is unlikely that PVR transcripts detected by in situ hybridization are not translated. Organ homogenates from PVR transgenic mice contain poliovirus binding activity (24), and freshly dispersed transgenic mouse kidney cells express the PVR on the cell surfaces, as shown by their ability to bind poliovirus. Virus may be unable to reach some cells which express PVR RNA, such as developing T lym-

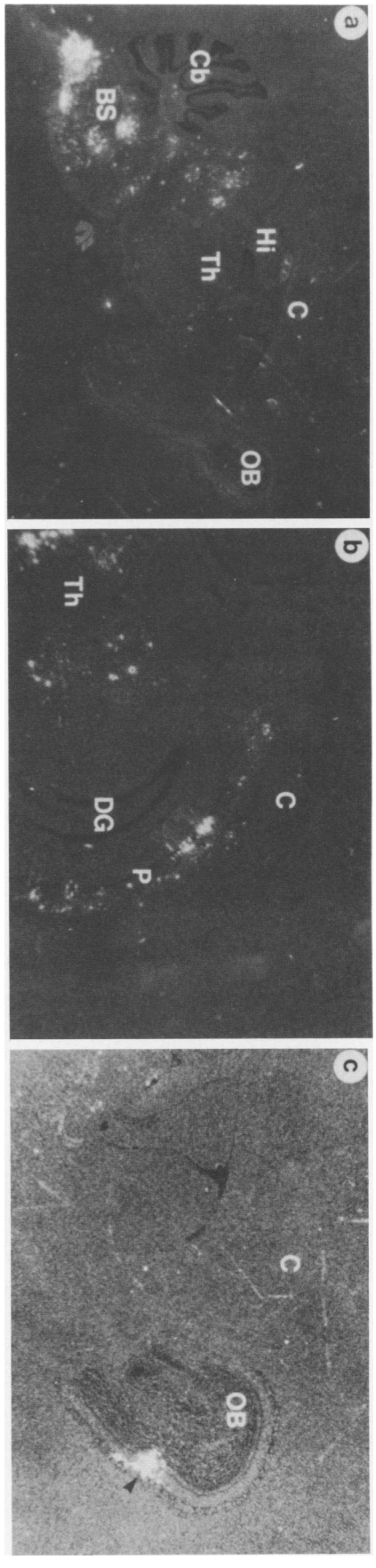


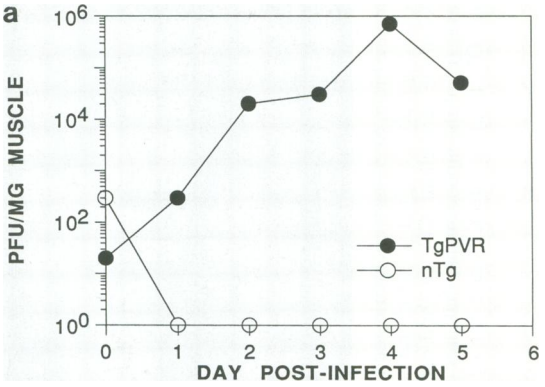
FIG. 4. In situ detection of poliovirus RNA in infected PVR transgenic mouse brain. Sections were hybridized with ^{35}S-labeled antisense P1/Mahoney viral RNA probe. Silver grains, indicating positive hybridization, appear bright white in the dark-field photomicrograph of sagittal (a and c) and coronal (b) sections of brain. (a) Strong hybridization signals localized specifically in the brain stem (BS). Infected neurons are in the deep nuclei of the cerebellum (Cb), cerebral cortex (C), hippocampus (Hi), thalamus (Th), and olfactory bulb (OB). Magnification, ×13. (b) Strong hybridization in neurons in the pyramidal layer (P) of the hippocampus, thalamus (Th), and cerebral cortex (C) but not in the dentate gyrus (DG) of the hippocampus. Magnification, ×27. (c) Higher magnification of olfactory bulb (OB) from the infected brain. Strong hybridization signals in a patch of neurons in the olfactory bulb. The cerebral cortex (C) is identified. Magnification, ×27.

phocytes of the thymus and epithelial cells of Bowman's capsule. However, tubular epithelial cells in the kidney and endocrine cells in the adrenal cortex should be exposed to circulating viruses. Poliovirus replication was not detected in these cells, indicating that poliovirus tissue tropism is not governed solely by expression of the PVR gene nor by accessibility of cells to virus.

Alternative splicing of PVR transcripts might control susceptibility of tissues to poliovirus infection. Human tissues contain both membrane-bound and secreted PVR isoforms, generated by alternative splicing of PVR mRNA (15). Expression of secreted PVRs in transgenic mouse tissues might result in neutralization of poliovirus infectivity (14). The results presented here, together with observations made with another PVR transgenic mouse line (16), reveal that RNAs encoding both membrane-bound and secreted PVRs are present in susceptible and nonsusceptible tissues, as is found in human tissues (15). These results, together with our observation that dispersed kidney cells in culture (from which soluble PVR would have been removed by washing) are still resistant to poliovirus infection, support the idea that secreted PVRs are not likely to restrict poliovirus in vivo. Alternatively, virus binding to nonsusceptible tissues might be blocked in vivo by the natural ligand of the PVR, or poliovirus entry might require factors in addition to the PVR that are lacking in nonsusceptible tissues. For example, expression of human CD4 in rodent cells is not sufficient to render these cells susceptible to human immunodeficiency virus type 1 infection because of a block at the level of entry (18).

Poliovirus replication in nonsusceptible tissues might be controlled at stages beyond virus entry, such as translation, replication, or assembly. This possibility has been generally discounted in the past, because inoculation of viral RNA intracerebrally into rabbits, chicks, guinea pigs, and hamsters results in one cycle of replication and production of infectious virus (10). However, these experiments indicate only that poliovirus host range restriction (species tropism) is determined at the level of entry in neural cells. Whether there is an internal block to poliovirus infection in nonsusceptible tissues remains unresolved. This question is being addressed by determining whether introduction of viral RNA into fresh dispersed kidney cells leads to poliovirus replication.

Although poliovirus infection in primates is restricted, cells from almost any tissue develop susceptibility to infection after cultivation in vitro (6, 9, 13). PVR transgenic mouse kidney cells express poliovirus binding sites but are initially resistant to poliovirus infection. When cultured in vitro, kidney cells develop susceptibility to poliovirus infec-



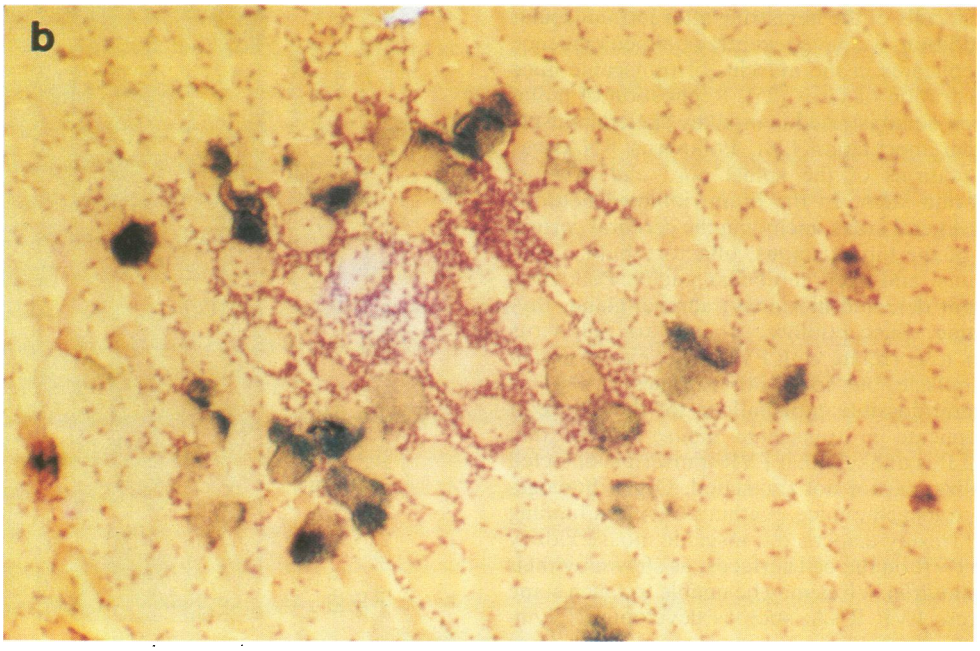


FIG. 5. Poliovirus replication in PVR transgenic mouse skeletal muscle. (a) Time course of poliovirus replication in skeletal muscle. Transgenic (TgPVR) or nontransgenic (nTg) mice were inoculated with 5×10^5 PFU of P1/Mahoney in the left hamstring muscle. At the indicated times, the hamstring was removed, homogenized, and the virus titer was determined by plaque assay. Each point represents the mean of values obtained for three mice. (b) In situ detection of poliovirus RNA in infected PVR transgenic mouse skeletal muscle. Cross-section prepared from hamstring of a paralyzed mouse inoculated intramuscularly as described for panel a. Section was hybridized with ^{35}S-labeled antisense P1/Mahoney viral RNA probe. Silver grains, indicating positive hybridization, appear bright green in the polarized light epiluminescence photomicrographs. Strong hybridization signals localized in muscle cells. Note extensive tissue infiltration in the infected area. Magnification, ×265.

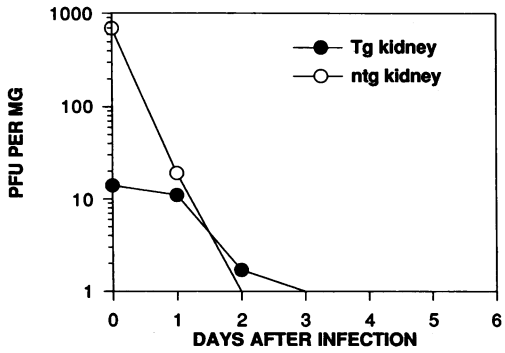


FIG. 6. Poliovirus replication in PVR transgenic mouse kidney. Transgenic (Tg) and nontransgenic (ntg) mice were inoculated intraperitoneally with 5×10^7 PFU of P1/Mahoney. Mice were sacrificed daily, and virus titer in kidney was determined by plaque assay on HeLa cell monolayers. Each time point represents the average of three mice.

tion after 24 h. The basis of the acquired susceptibility to poliovirus infection in these cells is not known, but might involve the induction of factors required for virus entry or replication. We are currently attempting to identify the changes that occur in cultured PVR transgenic mouse kidney cells that permit poliovirus infection. These results will provide information on the block to poliovirus infection in these cells and might reveal the mechanism of poliovirus tissue tropism in primates.

The sites of poliovirus replication in the CNS of PVR transgenic mice closely parallel those observed in primates, with one exception: in transgenic mice, pathological changes were observed in the hippocampus (24). The results reported here demonstrate that poliovirus replicates in the hippocampus of PVR transgenic mice, providing an explanation for the previously observed pathological changes. It is not clear why involvement of the hippocampus is not observed in primates. Indeed, it is interesting that in both transgenic mice and primates, poliovirus replication is limited to specific areas of the brain. Intracerebral inoculation of PVR transgenic mice resulted in viral replication at brain sites not observed after intraperitoneal inoculation, such as the olfactory bulb. These results suggest that the route of inoculation may determine which brain neurons become infected. However, it is not likely to be the only determinant of tropism, since even after intracerebral inoculation, replication was not observed in all brain neurons. Other determinants might include susceptibility of specific cell types to infection or physical restrictions to virus spread within the brain.

In this study, poliovirus replication was detected in neurons in both the ventral and dorsal horns of the spinal cord. However, inflammation and neuronal degeneration was localized largely to the ventral horns, suggesting that neurons in the dorsal horn were either infected at a late stage of the disease or that poliovirus replication in these neurons does not lead to cell destruction. Poliovirus histopathology is observed in the posterior horn (analogous to the dorsal horn of mice) in human and monkey spinal cord, although less frequently than in the anterior horn (4).

The mechanism by which poliovirus enters the CNS from the blood is of great interest. Two possibilities have been suggested which are not mutually exclusive: the virus crosses capillaries in the CNS or it enters the neuromuscular junction and spreads via nerve fibers to the CNS. Virus antigen has been detected by immunofluorescence in vascular endothelial cells of monkeys infected with poliovirus (2, 12), and PVR has been detected in a small percentage of freshly dispersed endothelial cells (5). These observations suggest that poliovirus may use receptors on endothelial cells to gain access to the CNS from capillaries. However, neither PVR expression nor virus replication was detected in endothelial cells of several PVR transgenic mouse organs, including the CNS. Poliovirus spread in transgenic mice is therefore not likely to involve receptors on endothelial cells. The presence of poliovirus RNA in axonal and dendritic processes of neurons suggests that virus could spread though neural pathways. In support of this hypothesis, preliminary data suggest that poliovirus spreads to the spinal cord through peripheral nerves after intramuscular inoculation of PVR transgenic mice (25).

An important question is whether studying poliovirus tropism in PVR transgenic mice provides information on tropism of the virus in primates. The pattern of expression of many human genes in transgenic mice is often similar to that observed in humans (reviewed in reference 20). Indeed, in both PVR transgenic mice (24) and humans (7, 19), the PVR gene is expressed in many tissues, suggesting that PVR expression in transgenic mice may mimic that in humans. One difference, however, is that poliovirus binding sites are expressed in all PVR transgenic mouse tissues examined (24), whereas binding sites in humans have been detected largely in neural tissues and intestine and occasionally in kidney and liver (9, 17). It is possible that the basis of poliovirus tropism in humans differs from that in PVR transgenic mice and is determined by factors that control the ability of the receptor to bind virus. Alternatively, poliovirus binding sites might be expressed in all human tissues, but at low levels or in unstable forms, making their detection difficult in some tissues. The PVR transgenic mice studied to date contain multiple copies of the PVR gene and express high levels of PVR mRNA, perhaps facilitating detection of binding sites in all organs. Resolution of this question awaits examination of PVR gene expression in human tissues by in situ hybridization for comparison with that in PVR transgenic mice and development of more-sensitive assays to detect poliovirus binding sites in human tissues. Preliminary results indicate that PVR expression in human placenta is restricted to specific cell types, suggesting that PVR in humans is expressed in a cell-specific pattern (25). It will be of interest to use PVR transgenic mice to determine how PVR gene expression is regulated in a cell-type-specific manner.

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