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Transgenic mice susceptible to poliovirus

(virus receptor/human receptor gene/central nervous system/attenuated strain)

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ABSTRACT **Poliovirus-sensitive transgenic mice were produced by introducing the human gene encoding cellular receptors for poliovirus into the mouse genome. Expression of the receptor mRNAs in tissues of the transgenic mice was analyzed by using RNA blot hybridization and the polymerase chain reaction. The human gene is expressed in many tissues of the transgenic mice just as in tissues of humans. The transgenic mice are susceptible to all three poliovirus serotypes, and the mice inoculated with poliovirus show clinical symptoms similar to those observed in humans and monkeys. Rabbit anti-poliovirus serum detects the antigens mainly in motor neurons in the anterior horn of the spinal cord and in nerve cells in the medulla oblongata and pons of the paralyzed transgenic mice. Therefore, cell types sensitive to poliovirus in the central nervous system of the transgenic mice appear to be identical to those of humans and monkeys. Furthermore, many more doses of oral poliovirus vaccine strains than of the virulent strains are required to cause paralysis in the transgenic mice. This may reflect the observation that the virulent strain multiplies more efficiently in the central nervous system than the attenuated strain. Thus, the transgenic mice may become an excellent new animal model to study molecular mechanisms of pathogenesis of poliovirus and to assess oral poliovirus vaccines.**

Poliovirus, the causative agent of poliomyelitis, is a human enterovirus that belongs to the Picornaviridae family and is classified into three stable serotypes (type 1, type 2, and type 3). Poliovirus infection is initiated by ingestion of virus followed by its primary multiplication in the oropharyngeal and intestinal mucosa. Extensive viral multiplication occurs in the tonsils and Peyer's patches of the ileum. From these sites, virus drains into deep cervical and mesenteric lymph nodes, and then into the blood. Although many tissues are exposed to the virus during the viremic phase, sites of poliovirus replication are limited to certain tissues. Paralytic poliomyelitis occurs as a result of destruction of motor neurons in the central nervous system (CNS). Poliovirus infects only primates and cannot infect mice except for type 2 virulent strains. Therefore, monkeys have to be used to investigate the pathogenesis of poliovirus as well as to test the quality of live attenuated poliovirus vaccines.

The characteristic species specificity and tissue tropism of poliovirus are considered to be primarily determined by a specific cell-surface receptor (1–3). Recently, the genomic and complementary DNAs for human poliovirus receptors (PVRs) were isolated from HeLa S3 cells (4, 5). In addition, four mRNA isoforms for human PVRs, that is, two membrane-bound (PVRα and PVRδ) and two secreted (PVRβ and PVRγ) forms, have so far been identified and shown to be generated by alternative splicing from the primary transcript (5). Of these, the membrane-bound PVRα and PVRδ, which correspond to H20A and H20B (4), are functional receptor molecules. Although it has been proved that PVRs are members of the immunoglobulin superfamily (4), physiological functions of these molecules are totally unknown at present.

Mouse L cells are not permissive for poliovirus infection. However, mouse L cell transformants carrying the human PVR gene (assigned the symbol *PVS* for poliovirus sensitivity) (5) or the PVR cDNA (4, 5) are permissive for the infection of all three serotypes of poliovirus. This observation confirms the previous notion that all three serotypes of poliovirus compete for the same cellular receptor (3). This result also indicates that mouse cells have cellular factors supporting poliovirus replication except for the receptor. This in turn led to the possibility for producing poliovirus-sensitive transgenic mice by introducing the human PVR gene into the mouse genome.

Here we describe the introduction of the human poliovirus receptor gene into the mouse genome and the characterization of the transgenic mice in regard to the expression of the human gene in mouse tissues and the permissiveness for poliovirus infection.

MATERIALS AND METHODS

Production of Transgenic Mice. The human PVR gene has been cloned into cosmid vector pTL5 and designated HC3 and HC5 (5). The cosmid clones were linearized by cutting at the unique *Sal* I site within the nucleotide sequence on the vector DNA. The linearized DNA was introduced into the pronuclei of ICR or C57BL/10 mouse zygotes as described by Brinster *et al.* (6).

Southern and RNA Blot Hybridization. The genomic DNAs were prepared from HeLa S3 cells and the liver of the mice and were digested with *Bam*HI or *Hin*dIII. Ten micrograms of the digests was separated by gel electrophoresis on 0.8% agarose and analyzed by Southern blot hybridization using standard procedures (7). Messenger RNAs of cells or tissues were isolated by the cesium trifluoroacetate/guanidine thiocyanate method (8). Two micrograms of poly(A)$^+$ RNA purified by Oligotex-dT30 (Takara Shuzo, Kyoto) was analyzed by RNA (Northern) blot hybridization (7).

An *Eco*RI/*Bam*HI cDNA fragment (nucleotide positions 278–1227), indicated by a line with arrowheads in Fig. 1*a*, was labeled with ^{32}P and used as a probe for both Southern and Northern hybridization experiments.

Polymerase Chain Reaction (PCR). PCR was used for screening the transgene in genomic DNA of possible transgenic mice. Two pairs of synthetic oligodeoxynucleotides specific for the human PVR gene sequence were synthesized

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Abbreviations: PVR, poliovirus receptor; CNS, central nervous system; pfu, plaque-forming units.

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and used as primers for PCR. One pair corresponds to nucleotide positions 142–161 and positions 350–369 of PVRα cDNA, and the other corresponds to positions 1829–1848 and positions 2209–2228 (see refs. 4 and 5 for nucleotide positions).

For the detection of isoforms of PVR mRNAs expressed in tissues (brain, spinal cord, heart, liver, kidney, and spleen) of the transgenic mice, cDNA was synthesized from 2 μg of the total RNA with a specific primer and then amplified as described (5).

Neurovirulence Tests. Virulent and attenuated poliovirus strains were grown in suspension-cultured HeLa S3 cells at 37°C and 35.5°C, respectively. Virus titers were measured by plaque assay using African green monkey kidney cells (9).

The transgenic mice of 6 to 7 weeks were inoculated intracerebrally with 30 μl each of poliovirus solutions at various titers. Animals were observed every 12 hr for paralysis or death up to 14 days after the inoculation.

Recovery of Viruses from the CNS. The amount of viruses in the CNSs of mice inoculated intracerebrally with the virulent Mahoney or attenuated Sabin 1 strains of type 1 poliovirus was measured on day 7 after the inoculation, when the transgenic mice inoculated with 10^3 plaque-forming units (pfu) of the Mahoney strain or 10^6 pfu of the Sabin 1 strain showed paralysis. On the same day, normal mice inoculated with the viruses were also sacrificed. The brain (average weight, 0.45 g) and spinal cord (average weight, 0.1 g) were separately homogenized with 10 ml of Eagle's minimal essential medium in a Potter–Elvehjem glass homogenizer (10 strokes). The homogenates were centrifuged at 3000 rpm (1500 × g) for 20 min, and virus titers in the supernatants were measured by plaque assay as described above.

Immunocytochemistry. The CNSs from the transgenic mice and normal mice inoculated intracerebrally with or without the Mahoney strain of poliovirus were fixed in 10% buffered formalin solution and embedded in paraffin. Thin sections (3-μm thickness), after deparaffinization, were treated with 0.25% trypsin for 2 hr and used for detection of viral antigens by the immunoperoxidase method (avidin–biotin complex, ABC). Hyperimmune anti-type 1 poliovirus rabbit serum was applied for the first overlay for 24 hr at 4°C. After washing, vectastain ABC kits (Vector Laboratories) were used for visualization.

RESULTS

Transgenic Mice Carrying the Human PVR Gene. The structures of the human PVR gene and mRNA for PVRα are shown in Fig. 1*a*. The cosmid clone HC3 or HC5 was linearized by digesting with *Sal* I and was introduced into the mouse genome. Mice were screened for the transgene by PCR with two pairs of primers. The positions are indicated by vertical arrows in Fig. 1*a*. As a result, we obtained three lines of transgenic mice (ICR-PVRTg1, ICR-PVRTg5, and ICR-PVRTg21) derived from strain ICR and one line (B10-PVRTg8) derived from strain C57BL/10. Transgenes in these lines are maintained in the hemizygous stage. The transmission rate of the PVR gene was approximately 50% for all of the lines of the transgenic mice. This suggests that the transgene integrates into a single locus of a certain chromosome of each strain. Further characterizations were performed with ICR-PVRTg1 mice carrying HC3.

Southern blot hybridization was carried out to detect the transgene in the transgenic mice ICR-PVRTg1 (Fig. 1*b*). As expected from the restriction map and position of the probe, radioactive bands were detected at positions of 10, 3.2, and 1.2 kilobases (kb) in *Bam*HI digests (lane 5) and at 7.5, 6, and 5 kb in *Hin*dIII digests (lane 6) of the liver DNA of the transgenic mice (Fig. 1*b*). The same patterns were obtained in the case of DNAs of cosmid clone HC3 (lanes 1 and 2) and

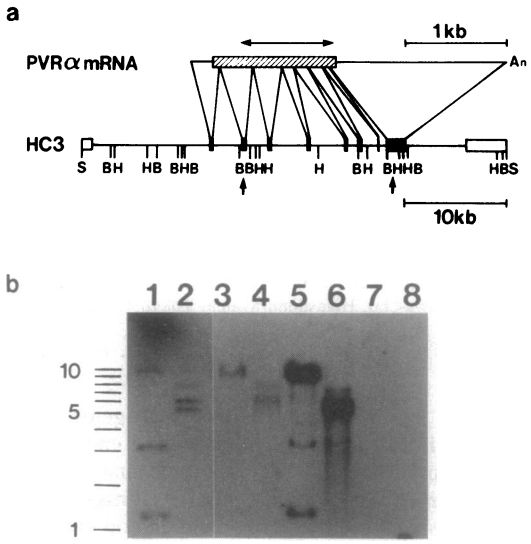

FIG. 1. The structures of the human PVR gene and PVRα mRNA (*a*) and Southern blot analysis of the transgene (*b*). (*a*) Structures of the human PVR gene cloned in cosmid vector pTL5 (HC3) and the mRNA for PVRα. Restriction sites of *Bam*HI, *Hin*dIII, and *Sal* I are indicated by B, H, and S, respectively. Filled boxes represent the exons of the PVR gene, open boxes represent the vector sequence, and a hatched box represents the translated sequence of PVRα mRNA. Scales for the length of the nucleotide sequences of PVRα mRNA and the PVR gene are shown above and below the structure, respectively. The positions of two pairs of primers for PCR are indicated by vertical arrows. A cDNA corresponding to the nucleotide sequence indicated by a line with arrowheads is a *Eco*RI/*Bam*HI fragment used as a probe in Southern blot hybridization in *b* and Northern blot hybridization in Fig. 2*a*. (*b*) Southern blot analysis of the transgene. Cloned PVR gene (lanes 1 and 2) or genomic DNA from HeLa S3 cells (lanes 3 and 4), the liver of the transgenic mice (ICR-PVRTg1) (lanes 5 and 6), or the liver of the normal mice (lanes 7 and 8) was digested with *Bam*HI (lanes 1, 3, 5, and 7) or *Hin*dIII (lanes 2, 4, 6, and 8). Ten micrograms of genomic DNA digests was separated by agarose gel electrophoresis and analyzed by Southern blot hybridization with a probe indicated in *a*. Positions of DNA fragments (1-kb DNA ladder, Bethesda Research Laboratories) used as size markers are indicated by lines with numbers in kbp on the left-hand side of the figure.

HeLa S3 cells (lanes 3 and 4). No band was detected in DNA digests of the liver of littermates with no transgene (lanes 7 and 8). These results suggest that the genome of the ICR-PVRTg1 carries the whole human PVR gene. From the intensity of radioactivity shown in Fig. 1*b*, the copy number of the transgene appears to be ≈10-fold that in HeLa S3 cells.

Expression of the Human PVR Gene in Mice. To investigate the expression of the human PVR gene in various tissues of the transgenic mice, Northern blot hybridization was performed to analyze PVR mRNAs in the brain, spinal cord, heart, liver, kidney, and spleen of the ICR-PVRTg1 mice. The results are shown in Fig. 2*a*. The cDNA probe detected human PVR mRNAs at a position corresponding to a length of ≈3.3 kb of poly(A)$^+$ RNA from all tissues except liver. Since PVR mRNAs are detected also in the liver by PCR as described below, the level of the mRNA must be very low in the liver of the transgenic mice. Apparent density of bands is different in the tissues. These results strongly suggest that the amount of PVR mRNAs varies from tissue to tissue. Thus expression of the human PVR gene appears in many tissues of the transgenic mice as it does in human tissues (4, 5).

Since mRNAs for both membrane-bound (functional) and secreted forms of PVRs migrate in a gel at the same position, we used PCR to know the type of PVR mRNAs expressed in individual tissues (Fig. 2*b*). Three bands corresponding to mRNA species for PVRα, PVRβ, and PVRγ were observed

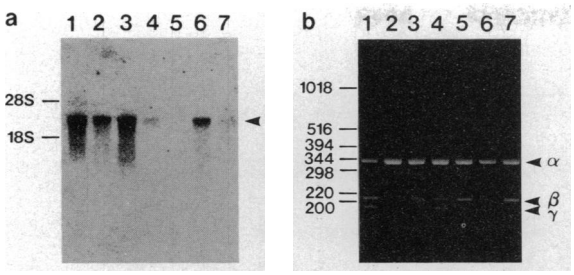

FIG. 2. Expression of PVR mRNAs in the transgenic mice. RNAs from HeLa S3 cells (lane 1), the brain (lane 2), spinal cord (lane 3), heart (lane 4), liver (lane 5), kidney (lane 6), and spleen (lane 7) were analyzed by Northern blot hybridization (*a*) and PCR (*b*). (*a*) Poly(A)$^+$ RNA from various tissues was analyzed. Positions of 28S and 18S rRNAs are indicated on the left and that of 3.3-kb PVR mRNA is indicated on the right by an arrowhead. (*b*) PVR mRNA isoforms expressed in various tissues were examined. Bands specific for mRNA isoforms for PVRα, PVRβ, and PVRγ are indicated by arrowheads. The length of DNA fragments used as size markers is indicated in numbers of base pairs on the left side of the figure.

in PCR products from RNAs of all tissues tested as well as HeLa S3 cells (Fig. 2*b*, lane 1). This observation is similar to that obtained with RNAs from human tissues (5). The results strongly suggest that a similar multiple splicing also occurs in many tissues of the transgenic mice as it does in those of humans and provides three different PVR mRNA isoforms in regard to the nucleotide sequences encoding the transmembrane domain. PVRδ mRNA was not detected in this experiment because of the primers used for the polymerization reactions.

Susceptibility to Poliovirus. Susceptibility of the transgenic mice to all three poliovirus serotypes was examined (Fig. 3 *a–c*). The virulent poliovirus strains Mahoney, Lansing, and Leon were used as representatives of types 1, 2, and 3, respectively. The transgenic mice were inoculated intracerebrally with various doses of these virus strains and observed for paralysis or death. The results are shown in Fig. 3 *a–c*. Doses of 10^2–10^3 pfu of the virulent strains caused death of the transgenic mice. However, normal ICR mice did not show any clinical symptoms even if 10^8 pfu of the Mahoney or Leon strain was inoculated (data not shown). Normal mice showed susceptibility only to the Lansing strain as expected (Fig. 3*e*) (10), although the level of the susceptibility was lower as compared with that of the transgenic mice. Thus, it is clear that the human PVR gene *PVS* confers the permissiveness for poliovirus to mice *in vivo* as well as mouse cells *in vitro*.

Inoculation of 10^7 pfu of the Mahoney strain with other routes such as intraperitoneal and subcutaneous routes also caused the same clinical symptoms and death as those with an intracerebral route. However, the transgenic mice orally administered with the Mahoney strain of up to 10^8 pfu did not show any clinical signs. Similar low rate of "virus take" from the alimentary canal is well known for cynomolgus monkeys that are highly sensitive to inoculation of poliovirus into the CNS.

Viral Multiplication Sites in the CNS. The main clinical sign observed in transgenic mice inoculated with poliovirus is flaccid paralysis of hindlimbs, which is similar to that of monkeys. This observation suggests that motor neurons in the spinal cord and nerve cells in the medulla oblongata are mainly damaged by the lytic virus infection. Accordingly, immunocytochemical analysis was performed on the CNS of the transgenic mice inoculated with the Mahoney strain intracerebrally (Fig. 4). Poliovirus antigens were detected mainly in motor neurons in any transverse section of the lumbar, thoracic, and cervical cords, medulla oblongata, and pons but not in the cerebellum or in cerebrum of the CNS of

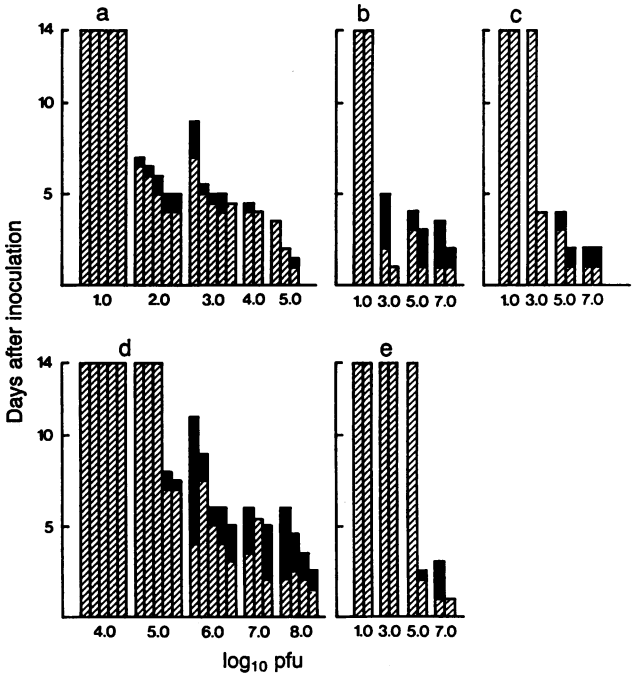

FIG. 3. Neurovirulence tests of poliovirus strains in transgenic mice. The transgenic mice were inoculated intracerebrally with the Mahoney strain (*a*), Lansing strain (*b*), Leon strain (*c*), and Sabin 1 strain (*d*) as described. Amounts of viruses inoculated per animal are indicated by values in $\log_{10}$ pfu at the bottom of each figure. Length of the vertical bars represents the life span of individual mice. Hatched bars and filled bars indicate survivors without any clinical symptoms and with paralysis, respectively. The results of the experiment with normal ICR mice (*e*) inoculated with the Lansing strain are also shown.

the transgenic mice inoculated with the Mahoney strain. The stained transverse sections from the cervical (Fig. 4 *a* and *b*) and lumbar (Fig. 4 *c* and *d*) cords are shown. However, transgenic mice without the virus infection and normal mice with the virus infection did not show any antigens or any lesions in the CNS (data not shown). These observations are very similar to the findings in humans who died of poliovirus infection at the acute stage and monkeys inoculated with the Mahoney strain into the spinal cord (data not shown). Thus, cell types sensitive to poliovirus infection in the CNS of the transgenic mice appear to be identical to those of humans and monkeys (11). Poliovirus antigens were also detected in similar cell types in the CNS of normal mice inoculated with the Lansing strain (12).

Neurovirulence of the Attenuated Strains. Neurovirulence of the live attenuated poliovirus vaccine strains was examined in the transgenic mice and compared with that of the virulent strains (Fig. 3). A dose of 10^6 pfu of the Sabin 1 strain was required to cause paralysis and death in all of the transgenic mice, whereas 10^2 pfu of the Mahoney strain was enough to kill the mice (compare Fig. 3 *a* and *d*). Furthermore, an average period of paralysis caused by the Sabin 1 strain was much longer than that by the Mahoney strain (Fig. 3 *a* and *d*). Similar observations were obtained when neurovirulence phenotypes of the Sabin 2 and Sabin 3 strains were compared with those of the corresponding virulent Lansing and Leon strains, respectively. A dose of 10^9 pfu of Sabin 2 strain caused paralysis but the mice survived up to 14 days. A dose of 10^8 pfu of Sabin 3 strain was necessary to cause paralysis and death in the transgenic mice (data not shown).

To know the reason for the difference in neurovirulence between the virulent and attenuated poliovirus strains in the transgenic mice, the viral multiplication of the Mahoney and Sabin 1 strains in the CNS was investigated and compared

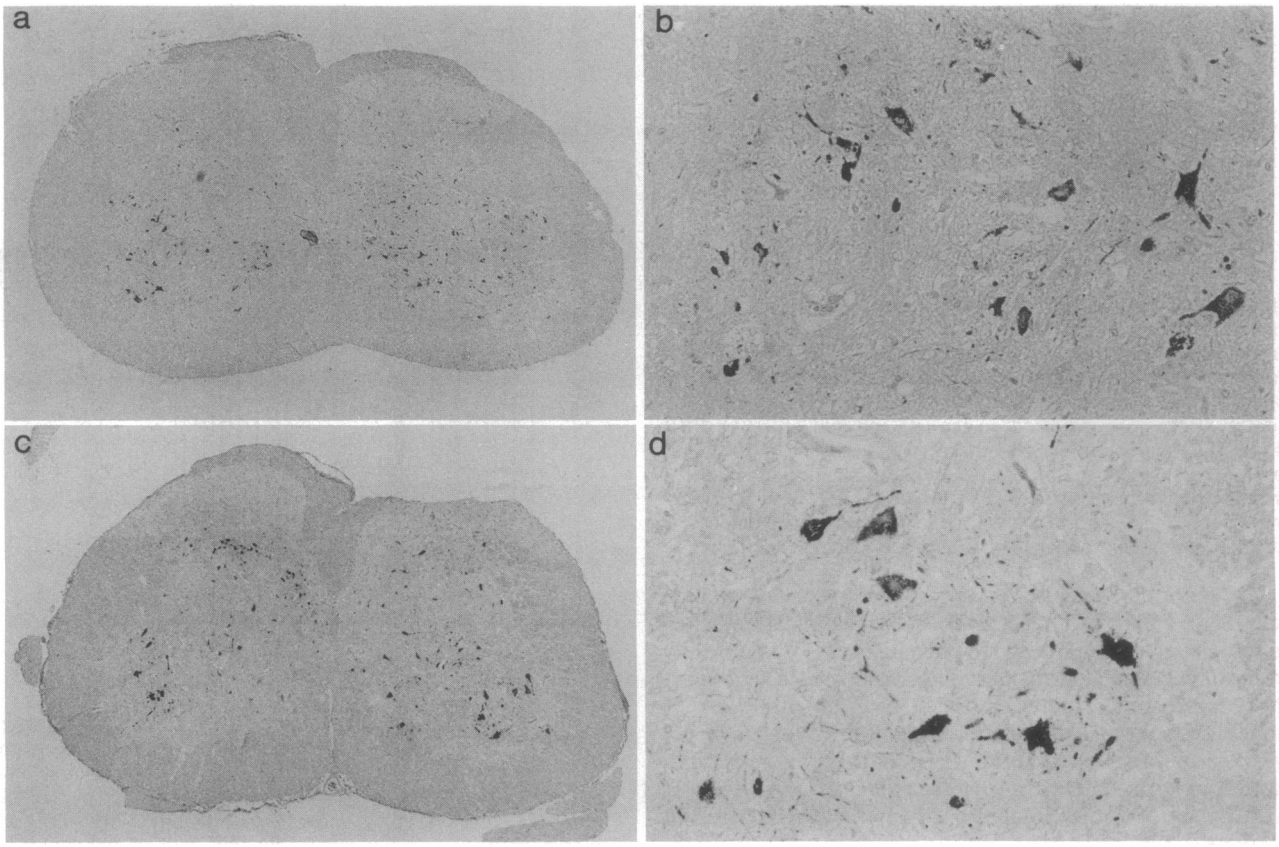

FIG. 4. Distribution of poliovirus antigens in the spinal cord of the transgenic mice. Thin sections were prepared from the CNS of the transgenic mice and stained as described. (*a*) Poliovirus antigens in the cervical cord. (*b*) Poliovirus antigens in motor neurons of the anterior horn of the cervical cord. (*c*) Poliovirus antigens in the lumbar cord. (*d*) Poliovirus antigens in the motor neurons of the lumbar cord. (*a* and *c*, ×13; *b* and *d*, ×65.)

with each other (Table 1). On day 7 after the inoculation, the transgenic mice inoculated with 10^3 pfu of the Mahoney strain or 10^6 pfu of the Sabin 1 strain showed paralysis. Viruses were recovered from the CNS of these mice and the amounts of viruses were measured. At the same time, normal ICR mice inoculated with 10^3 or 10^6 pfu of the Mahoney strain or 10^6 pfu of the Sabin 1 strain were sacrificed, and the amounts of viruses recovered from the CNSs were also measured.

As shown in Table 1, the amounts of both the Mahoney and Sabin 1 strains dramatically decrease in the CNS of normal mice during the 7 days after the inoculation, although the amount of the Mahoney strain recovered is approximately 100-fold of that of the Sabin 1 strain, suggesting that the Mahoney strain is more stable than the Sabin 1 strain in the CNS of the mice. In the case of the transgenic mice, however, $\approx 10^7$ pfu of the virus was recovered from both the brain and spinal cord of the mice inoculated with 10^3 pfu of the Mahoney strain, and a considerable amount of virus was recovered from the CNS of the mice inoculated with 10^6 pfu of the Sabin 1 strain, although no virus was detected in the CNS inoculated with 10^3 pfu of the Sabin 1 strain (Table 1). The amount of the virus recovered was almost 1/10th of the Sabin 1 strain virus inoculated but almost 10^4-fold of the Mahoney strain virus inoculated (Table 1). The data clearly indicate that the multiplication ability of the Sabin 1 strain in the CNS is much lower than that of the Mahoney strain. These results strongly suggest that the different mouse neurovirulence between the two strains is due to different efficiency in the viral multiplication in the CNS. Similar observations were reported for the CNS of monkeys (11). Thus, the results of the mouse neurovirulence tests appear to reflect those of monkey neurovirulence tests, as suggested by mouse neurovirulence tests on mouse adapted chimera polioviruses that carry a part of the viral capsid polyprotein derived from the Lansing strain (13, 14).

Table 1. Poliovirus multiplication in the CNS of mice

Mice	Poliovirus strain	Amount of virus inoculated, $\log_{10}$ pfu	Amount of virus recovered, $\log_{10}$ pfu	
			Brain	Spinal cord
Normal	Mahoney	3	ND	ND
		6	2.7	2.3
	Sabin 1	6	1.0	ND
Transgenic	Mahoney	3	6.8	7.1
	Sabin 1	3	ND	ND
		6	4.9	5.3

Poliovirus strains were prepared and inoculated into mice as described in *Materials and Methods*. Two or three mice were used in each case. ND, not detected.

DISCUSSION

Poliovirus-sensitive transgenic mice were produced by introducing the human PVR gene into the mouse genome. Northern blot hybridization analysis and PCR experiments indicated that the human PVR gene introduced into the mouse genome transcribed in a number of tissues of the transgenic mice, and that the alternative splicing observed in human tissues also occurred in the mouse tissues. The observations suggest that the control mechanisms for the expression of the human PVR gene in the transgenic mice are the same as those in humans, although the expression of the PVR gene in individual cell types is not studied yet. Virus multiplication

sites in the CNS of the transgenic mice appear to be identical to those in the CNSs of humans and monkeys. This result may indicate that the same control mechanisms for the human PVR gene expression exist at least in the CNSs of the transgenic mice and humans.

The reason why all of the human tissues in which the PVR gene is expressed are not the sites of poliovirus replication is not clear. It is possible that the functional PVRs are produced only in the limited cell types that contribute to the development of pathogenesis of the virus. For example, endothelial cells carrying PVR are proposed as the extraneural target cells that may have a role in the establishment of the persistent viremia (15). Alternatively, other factors, in addition to PVR, may also have important roles to provide the permissiveness for poliovirus. Such a factor may be a 100-kDa polypeptide identified by Shepley *et al.* (16). More studies may give an insight into the mechanisms for pathogenesis of poliovirus in humans if the control mechanisms for the human PVR gene expression in the transgenic mice are the same as those in humans.

The molecular mechanisms for the differential multiplication between the virulent and attenuated poliovirus strains are unknown at present. Monkey neurovirulence tests on a number of recombinant viruses between the virulent Mahoney and attenuated Sabin 1 strains have revealed that relatively strong determinant(s) influencing the neurovirulence or attenuation phenotype resided in the 5′ noncoding region of the genome (9, 17, 18). These observations suggest that cellular factor(s) supporting poliovirus replication by recognizing the structure of the 5′ noncoding sequence of the genome is not sufficiently functional for the replication of the Sabin 1 strain in the CNS of monkeys. Although the mechanisms for the differential multiplication might not be the same in the CNSs of monkeys and the transgenic mice, it is of interest that the difference in multiplication of the virulent and attenuated poliovirus strains is similarly observed in the transgenic mice. It is possible therefore that the transgenic mice could become an animal model in place of monkeys to assess oral poliovirus vaccines.

Physiological function of the PVR molecules is unknown at present. The immunoglobulin superfamily is one of the important groups not only in immunity but also in the mediation of cell-surface recognition to control the behavior of cells in various tissues (19). Elucidation of cell type-specific expression of the human PVR gene depending on a stage of differentiation of cells and tissues of the transgenic mice may give an insight into the physiological functions of the molecules.

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1. Holland, J. J., McLaren, J. C. & Syverton, J. T. (1959) *J. Exp. Med.* **110**, 65–80.
2. Holland, J. J. (1961) *Virology* **15**, 312–326.
3. Crowell, R. L. & Landau, B. J. (1983) *Compr. Virol.* **18**, 1–42.
4. Mendelsohn, C. L., Wimmer, E. & Racaniello, V. R. (1989) *Cell* **56**, 855–865.
5. Koike, S., Horie, H., Ise, I., Okitsu, A., Yoshida, M., Iizuka, N., Takeuchi, K., Takegami, T. & Nomoto, A. (1990) *EMBO J.* **9**, 3217–3224.
6. Brinster, R. L., Chen, H. Y., Trumbauer, M. E., Yagle, M. K. & Palmiter, R. D. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4438–4442.
7. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
8. Okayama, H., Kawaichi, M., Brownstein, M., Lee, F., Yokota, T. & Arai, K. (1987) *Methods Enzymol.* **154**, 3–28.
9. Kawamura, N., Kohara, M., Abe, S., Komatsu, T., Tago, K., Arita, M. & Nomoto, A. (1989) *J. Virol.* **63**, 1302–1309.
10. LaMonica, N., Meriam, C. & Racaniello, V. R. (1986) *J. Virol.* **57**, 515–525.
11. Couderc, T., Christodoulou, C., Kopecka, H., Marsden, S., Taffs, L. F., Crainic, R. & Horaud, F. (1989) *J. Gen. Virol.* **70**, 2907–2918.
12. Jubelt, B., Gallez-Hawkins, G., Narayan, O. & Johnson, R. T. (1980) *J. Neuropathol. Exp. Neurol.* **39**, 138–148.
13. LaMonica, N., Almond, J. W. & Racaniello, V. R. (1987) *J. Virol.* **61**, 2917–2920.
14. Martin, A., Wychowsky, C., Couderc, T., Crainic, R., Hogle, J. & Girard, M. (1988) *EMBO J.* **7**, 2839–2847.
15. Couderc, T., Barzu, T., Horaud, F. & Crainic, R. (1990) *Virology* **174**, 95–102.
16. Shepley, M. P., Sherry, B. & Weiner, H. L. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7743–7747.
17. Omata, T., Kohara, M., Kuge, S., Komatsu, T., Abe, S., Semler, B. L., Kameda, A., Itoh, H., Arita, M., Wimmer, E. & Nomoto, A. (1986) *J. Virol.* **58**, 348–358.
18. Kohara, M., Abe, S., Komatsu, T., Tago, K., Arita, M. & Nomoto, A. (1988) *J. Virol.* **62**, 2828–2835.
19. Williams, A. F. & Berclay, A. N. (1988) *Annu. Rev. Immunol.* **6**, 381–405.