JOURNAL OF VIROLOGY, July 1997, p. 4915–4920 Vol. 71, No. 7
0022-538X/97/$04.00+0

Expression of the Poliovirus Receptor in Intestinal Epithelial Cells Is Not Sufficient To Permit Poliovirus Replication in the Mouse Gut

SUHUA ZHANG AND VINCENT R. RACANIELLO*

Department of Microbiology, Columbia University College of Physicians & Surgeons, New York, New York 10032

Received 29 January 1997/Accepted 3 April 1997

Although the initial site of poliovirus replication in humans is the intestine, previously isolated transgenic mice which carry the human poliovirus receptor (PVR) gene (TgPVR mice), which develop poliomyelitis after intracerebral inoculation, are not susceptible to infection by the oral route. The low levels of PVR expressed in the TgPVR mouse intestine might explain the absence of poliovirus replication at that site. To ascertain whether PVR is the sole determinant of poliovirus susceptibility of the mouse intestine, we have generated transgenic mice by using the promoter for rat intestine fatty acid binding protein to direct PVR expression in mouse gut. Pvr was detected by immunohistochemistry in the enterocytes and M cells of transgenic mouse (TgFABP-PVR) small intestine. Upon oral inoculation with poliovirus, no increase in virus titer was detected in the feces of TgFABP-PVR mice, and no virus replication was observed in the small intestine, although poliovirus replicated in the brain after intracerebral inoculation. The failure of poliovirus to replicate in the TgFABP-PVR mouse small intestine was not due to lack of virus binding sites, because poliovirus could attach to fragments of small intestine from these mice. These results indicate that the inability of poliovirus to replicate in the mouse alimentary tract is not solely due to the absence of virus receptor, and other factors are involved in determining the ability of poliovirus to replicate in the mouse gut.

Poliovirus, the causative agent of paralytic poliomyelitis, is a member of the picornavirus family, a group of small, icosahedral, positive-stranded RNA viruses (20). Poliovirus infection begins when the virus binds to the poliovirus receptor, Pvr, on the cell surface, followed by release of viral RNA into the cytoplasm (reviewed in reference 15). In susceptible hosts, poliovirus infection begins with ingestion of the virus followed by replication in the alimentary tract and spread to the blood (1, 21). During the viremic phase, poliovirus has access to many tissues, yet viral replication is detected only at a few sites, such as the central nervous system (CNS). The mechanism for this restriction is not known. Early work suggested that the cellular receptor might be a major determinant of poliovirus tissue tropism (9). However, recent data indicate that PVR is expressed in susceptible and nonsusceptible human tissues (4, 6, 13). The absence of poliovirus replication in PVR-expressing cells might be due to their inaccessibility to virus or inability to support viral replication (15, 17).

The identity of the primary site of poliovirus replication in the intestine remains a matter of debate. The detection of poliovirus in Peyer's patches of the ileum of infected chimpanzees led to the suggestion that poliovirus initially replicates in lymphocytes (1). However, it is not known whether virus is produced in the Peyer's patches or spreads there from other sites. In other studies, poliovirus was isolated from both the ileal wall and Peyer's patches of infected chimpanzees (21, 22), leading to the suggestion that poliovirus first replicates in epithelial cells and then spreads to the Peyer's patches. Poliovirus replication in the squamous epithelial cells of the oropharynx has also been reported (10). The epithelial lining of the small intestine consists of enterocytes, which comprise the first layer of cells in most of the intestinal lumen, and M cells, which are found above the Peyer's patches and are capable of transporting molecules from the intestinal lumen to the underlying lymphoid cells (5).

Transgenic mice that express the human PVR gene (TgPVR mice) have been identified as a model for studying poliovirus neurovirulence and pathogenesis (11, 18). After inoculation with poliovirus by the intracerebral, intraspinal, intravenous, or intramuscular route, TgPVR mice develop paralytic poliomyelitis. However, unlike the case for poliovirus infection in humans, poliovirus replication is not detected in the alimentary tract of TgPVR mice after oral inoculation. The absence of poliovirus replication in the TgPVR mouse alimentary tract might be due to the low levels of PVR expression in the small intestine (17, 19). In contrast, PVR RNA is readily detected in human enterocytes (19).

To determine whether Pvr is the sole determinant of poliovirus susceptibility in the mouse intestine, we have generated new transgenic mice (TgFABP-PVR mice) in which high levels of Pvr are expressed in M cells and enterocytes. PVR expression in TgFABP-PVR mice is under the control of the promoter from the rat intestine fatty acid binding protein (I-FABP) gene, which has been shown to direct expression of a reporter gene specifically in the enterocytes of transgenic mice (26). After oral inoculation of TgFABP-PVR mice with poliovirus, no viral replication was detected in the alimentary tract, as determined by titration of virus in the feces and in tissues of the small intestine. The inability of poliovirus to replicate in the TgFABP-PVR mouse small intestine was not due to the absence of virus binding sites. These results indicate that lack of Pvr expression is not the sole determinant of the resistance of the mouse intestine to poliovirus replication. Other factors may influence the ability of poliovirus to enter or replicate in cells of the mouse alimentary tract.

* Corresponding author. Mailing address: Department of Microbiology, Columbia University College of Physicians & Surgeons, 701 W. 168th St., New York, NY 10032. Phone: (212) 305-5707. Fax: (212) 305-5106. E-mail: racaniello@cuccfa.ccc.columbia.edu.

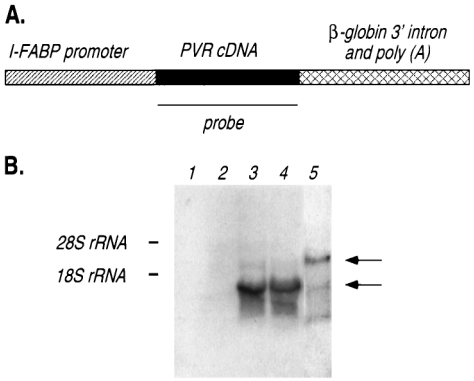

FIG. 1. (A) Structure of DNA used to generate transgenic mice. The 1.2-kb I-FABP DNA fragment ranged from nucleotides −1178 to +28 (where nucleotide 1 is the transcription start site) (26). The PVR cDNA was from nucleotides −120 to +1202 (where nucleotide 1 is the first base of the initiating AUG codon) (13). The human β-globin fragment (1.7 kb) begins at the third exon, codon 121, and includes the 3′ intron and polyadenylation signal (12). (B) Northern hybridization analysis of total cell RNA from the small intestine of normal (lane 1), TgPVR (lane 2), TgFABP-PVR2 (lane 3), and TgFABP-PVR3 (lane 4) mice and from the brain of TgPVR mice (lane 5). All lanes contained equal amounts of RNA, as judged by ethidium bromide staining. The DNA probe was the coding region of PVR shown in panel A. Lane 5 is from an autoradiograph that had been exposed twice as long as lanes 1 to 4, due to the low signal. The top arrow indicates the expected position of the PVR RNA produced in TgPVR mice, and the bottom arrow indicates the expected position of the PVR RNA produced in TgFABP-PVR mice. The autoradiograph was scanned by using Adobe Photoshop and lettered by using Canvas 5.0 (Deneba Software).

MATERIALS AND METHODS

Cells, virus, and antibody. HeLa S3 cells were grown in suspension cultures in Joklik minimal essential medium containing 5% horse serum and 10 μg of gentamicin per ml. For plaque assays, HeLa cells were plated in Dulbecco modified Eagle medium containing 5% bovine calf serum and gentamicin. Mouse L cells were maintained in Dulbecco modified Eagle's medium containing 10% bovine calf serum, 100 U of penicillin per ml, and 100 μg of streptomycin per ml.

Poliovirus strain P1/Mahoney was derived from the infectious cDNA clone (16) and propagated on HeLa cells. Hybridoma cells that secrete 711C, a mouse monoclonal antibody directed against the first domain of human PVR (14), were maintained in BM hybridoma medium with 2% fetal bovine serum.

Construction of a plasmid containing PVR cDNA under the control of the I-FABP promoter. The coding region of 20B PVR cDNA (13) was amplified by PCR, using a primer that created a *Bam*HI restriction enzyme site at the 3′ end. The 3′ noncoding region of 20B cDNA was not included because of the presence of a putative RNA destabilization sequence (13). This PCR product was then cloned into pBluescript (Stratagene) along with the 1.2-kb *Eco*RI/*Xba*I fragment of the promoter for rat I-FABP by three-way ligation. A 1.7-kb *Bam*HI/*Pst*I DNA fragment containing the human β-globin 3′ intron and poly(A) tract (12) was inserted downstream of the PVR coding region, to increase transcriptional efficiency in transgenic mice (3). The structure of the final plasmid, pFABP-PVR, was confirmed by restriction enzyme digestion and by sequencing the junctions created by ligation. For microinjection, pFABP-PVR was cleaved with *Hin*dIII and *Pst*I to remove vector sequences. The 4.2-kb insert was purified by agarose gel electrophoresis and dialyzed against 3 mM Tris HCl (pH 8.0)–0.3 mM EDTA. Purified DNA was diluted to 4 to 5 μg per ml before microinjection.

DNA transient transformation and virus infection. Mouse Ltk$^-$ cells were seeded in plastic cell culture plates 1 day before use at 2×10^6 cells per 6-cm-diameter plate. Plates were treated with 0.5 ml of a DNA-calcium phosphate coprecipitate consisting of 10 μg of cDNA and 10 μg of herring sperm DNA. After 20 h of incubation at 37°C, the medium was replaced, and incubation was continued for an additional 24 h. Cells were then infected with poliovirus at a multiplicity of infection of 0.1. After 45 min of adsorption at 37°C, the plates was washed three times with phosphate-buffered saline (PBS) containing 0.2% horse serum to remove the unattached virus, and medium was replaced. Aliquots of supernatant were removed at different times after infection, and virus titers were determined by plaque assay on HeLa cell monolayers.

Microinjection and production of transgenic mice. Fertilized eggs of the F_1 generation of C57BL6/J × CBA/J mice were isolated from the swollen ampulla of the oviduct of female mice that had mated with stud males the night before (8). Microinjection was performed with a Leitz micromanipulator attached to a Zeiss Axiovert microscope. Transgenic mouse founders were identified by PCR assay of tail DNA, using primers that correspond to the 20B PVR cDNA sequence. Southern blot analysis was also performed to ensure the integrity of the transgene and for the purpose of estimating the copy number. The copy number for TgFABP-PVR lines ranged between 10 and 20 copies of the transgene. To produce TgFABP-PVR × TgPVR hybrid mice, mice homozygous for the FABP-PVR transgene were crossed with homozygous TgPVR mice.

RNA and DNA isolation. Total RNA was isolated from small intestine by homogenization in 4 M guanidinium thiocyanate with a Brinkmann Polytron homogenizer followed by acidic phenol extraction. Isolated RNA was dissolved in diethylpyrocarbonate-treated water containing 0.5% sodium dodecyl sulfate and 1 mM EDTA (pH 8.0). Tails of 3-week-old mice were cut and digested in 50 mM Tris (pH 8.0), 0.5% sodium dodecyl sulfate, and 500 μg of proteinase K per ml. Undigested debris was removed by centrifugation. DNA was precipitated with isopropanol and dissolved in distilled water.

Antibody purification and biotinylation. Hybridoma cells secreting the anti-PVR monoclonal antibody 711C were grown in 15-cm-diameter plates in serum-free medium HB 101 (Irvine Scientific). Supernatants were collected and passed through a protein A affinity column (Bio-Rad). The column was then washed and eluted under conditions recommended by the manufacturer. For biotinylation, purified 711C antibody was dialyzed against 0.1 M sodium bicarbonate (pH 8.4) and diluted to 1 mg/ml; 0.5 mg of purified antibody was incubated with 30 or 45 μg of NHS-LC-Biotin (Pierce) at room temperature for 4 h. Free biotin was removed by a G25 column. The biotinylated antibody was tested by cell flow cytometry.

Immunohistochemistry. Mouse intestine was fixed in 4% paraformaldehyde and embedded in Tissue-Tek O.C.T. (Miles Inc.). Frozen sections (15 μm) were cut on a cryostat and placed on Fisher Superfrost tissue section slides. Tissue sections were air dried for 2 h, and the remaining Tissue-Tek O.C.T. was removed by washing with PBS. Approximately 0.2 μg of biotinylated 711C was added to each slide. Staining was carried out at 4°C overnight followed by staining with avidin β-galactosidase conjugate (Vector Laboratories) for 1.5 h at room temperature. After three washes with PBS, β-galactosidase activity was assayed by incubation with 0.05% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) in PBS with 3 mM $K_4Fe(CN)_6$, 3 mM $K_3Fe(CN)_6$, 1 mM $MgCl_2$, and 0.1% bovine serum albumin at room temperature overnight.

Oral and intracerebral inoculation of mice. Three-week-old transgenic and nontransgenic mice were orally inoculated with 0.1 ml of poliovirus by using an animal feeding needle (Popper & Sons) inserted into the esophagus. The detection of virus present in the feces at 12 to 16 h postinfection was used to confirm that the inoculum had been correctly placed. Animals were observed daily for death or paralysis for 18 days after inoculation.

Virus replication in mouse intestine. To monitor poliovirus replication in the mouse alimentary tract, feces were collected or the entire small intestine was removed at various times after oral inoculation. Fecal pellets were hydrated in PBS containing 0.2% horse serum and centrifuged in an Eppendorf Microfuge for 5 min. The small intestine was homogenized with a Brinkmann Polytron. Virus titers in fecal supernatants and intestinal homogenates were determined by plaque assay on HeLa cell monolayers.

PVR binding activity in mouse small intestine. The duodenum and jejunum were removed and cut open, and the contents were removed by washing with PBS. Tissues were cut with scissors into 5-mm-long pieces. Four to five pieces were incubated with 5×10^6 PFU of poliovirus in 500 μl of PBS at room temperature with rotation. After incubation for 2 h, the mixture was centrifuged in a microcentrifuge for 5 to 10 min. Virus titers in the supernatant were determined by plaque assay on HeLa cell monolayers.

RESULTS

Generation of transgenic mice. To direct PVR expression to the intestine of transgenic mice, the promoter for rat I-FABP was chosen. The I-FABP gene product plays a role in fatty acid uptake by intestinal epithelial cells and is a marker for differentiated, villus-associated, small intestine epithelial cells (25).

TABLE 1. Yields of poliovirus after infection of mouse L cells transiently transformed with PVR cDNA clones

Transforming DNA[a]	PFU/ml[b] at:	
	0 h p.i.	24 h p.i.
pBluescript	20	5
pFABP-PVR1	45	2.0×10^5
pFABP-PVR2	45	2.0×10^5
pSVL-20B	25	4.0×10^5

[a] L cells were transformed with the indicated plasmids and 48 h later infected with poliovirus type 1.

[b] Virus titers were determined by plaque assay of cell culture supernatants on HeLa cell monolayers. p.i., postinfection.

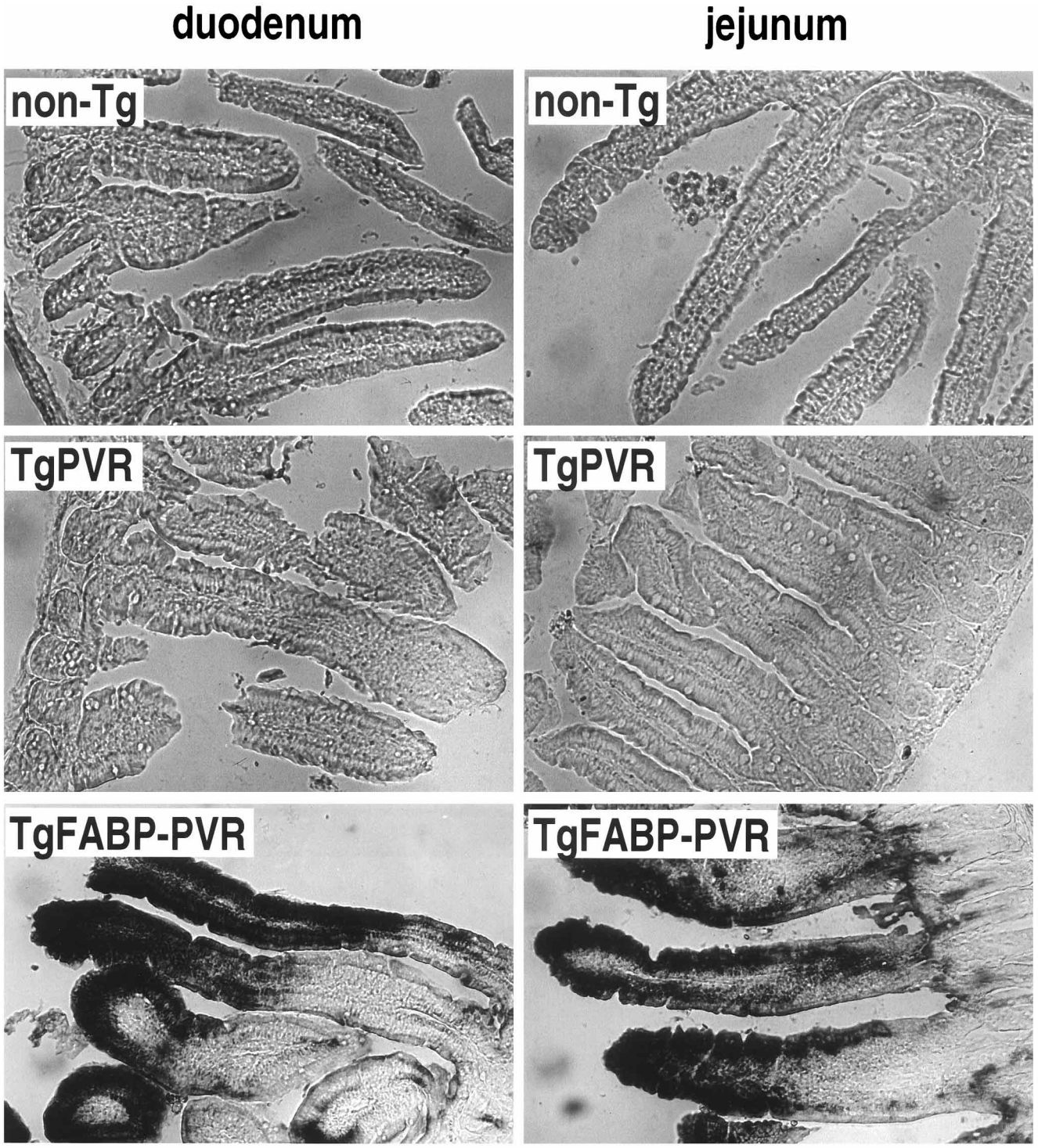

FIG. 2. Immunohistochemistry of mouse small intestine. Tissue sections were stained with anti-PVR monoclonal antibody 711C conjugated to biotin and then with avidin–β-galactosidase. Slides were viewed on a Leitz microscope under visible light at a magnification of ×100. Sections from duodenum and jejunum from normal, TgPVR, and TgFABP-PVR mice are labeled. Cells that express Pvr stain blue, which appears dark in the black-and-white image. Sections were photographed on slide film, digitized on a Polaroid CoolScan, converted to black and white in Adobe Photoshop, and lettered in Canvas 5.0 (Deneba Software). Final magnification, ×96.

The I-FABP promoter has been shown to direct high-level expression of a reporter gene in enterocytes of transgenic mouse small intestine (26). The coding region of PVR cDNA was cloned downstream of the I-FABP promoter (Fig. 1A). In addition, a fragment containing the human β-globin 3′ intron and poly(A) tract was placed immediately downstream of the PVR coding sequence to ensure proper posttranscriptional modification and mRNA stability. This construct was named pFABP-PVR.

To ensure that pFABP-PVR DNA will produce a functional

TABLE 2. Susceptibility of mice to inoculation with poliovirus type 1

Route of inoculation	Mouse[a]	PFU inoculated	No. paralyzed/ no. inoculated
Oral	TgFABP-PVR × TgPVR	2×10^8	0/6
	TgFABP-PVR +/+	2×10^8	0/6
	TgPVR	2×10^8	0/6
	Nontransgenic	2×10^8	0/6
Intracerebral	TgFABP-PVR +/+	2×10^8	5/5
	TgFABP-PVR +/+	2×10^7	1/5
	TgFABP-PVR +/−	2×10^8	1/5
	Nontransgenic	2×10^8	0/5

[a] ++ and +/− denote mice homozygous and heterozygous, respectively, for the transgene.

TABLE 3. Fecal titers of poliovirus after oral inoculation of mice

Mouse[a]	PFU/g of feces[b]	
	Day 0.5 (10^5)	Day 1.5 (10^3)
Nontransgenic	3.5	7.5
TgFABP-PVR	2.0	3.0
TgFABP-PVR × TgPVR	2.5	3.5

[a] Each mouse was given 10^7 PFU of poliovirus via an oral feeding needle inserted into the esophagus.

[b] Average obtained from three mice. In all cases, titers at days 4.5 and 7.5 were <50 PFU/g of feces.

poliovirus receptor, plasmid DNA was transiently transformed into receptor-negative mouse L cells followed by poliovirus infection. Infectious poliovirus was produced in L cells transformed with pFABP-PVR or pSVL-20B(PVR) cDNA but not in L cells transformed with vector DNA (Table 1).

The DNA insert from pFABP-PVR was used to generate transgenic mice, which were screened for the presence of the transgene by PCR analysis of tail DNA. Five transgenic founders (TgFABP-PVR mice) were identified and used for further study.

Expression of PVR in small intestine. Northern blot hybridization analysis was used to determine whether PVR RNA is expressed in the small intestine of TgFABP-PVR transgenic mice. High levels of a 1.4-kb PVR RNA were detected in the small intestine of offspring of two founder mice, TgFABP-PVR2 and TgFABP-PVR3 (Fig. 1B). This size is consistent with that predicted from the structure of pFABP-PVR. PVR RNA was not detected in the small intestine of either nontransgenic mice or previously isolated TgPVR mice that carry the human PVR gene (18) and was detected at low levels in the brain of TgPVR mice (Fig. 1B).

Immunohistochemistry was used to determine whether Pvr is expressed in the small intestine of TgFABP-PVR mice. PVR protein expression was detected at high levels in the duodenum and jejunum of the small intestine (Fig. 2) and at much lower levels in the ileum and colon (27). As expected from the previously described specificity of the I-FABP promoter, the staining is restricted to the epithelium (26). Pvr expression was also observed in M cells of the duodenum and jejunum (27). Pvr expression was not detected in the small intestine of nontransgenic and TgPVR mice (Fig. 2).

Susceptibility of mice to oral poliovirus inoculation. To determine whether expression of Pvr in the mouse small intestine confers susceptibility to poliovirus infection, TgFABP-PVR mice were crossed with TgPVR mice, which are susceptible to poliovirus infection by all routes except oral inoculation (18). TgFABP-PVR × TgPVR mice were orally inoculated with high titers (2×10^8 PFU) of poliovirus. No paralysis was observed up to 18 days after infection of TgFABP-PVR × TgPVR, homozygous TgFABP-PVR, TgPVR, or nontransgenic mice (Table 2). Oral susceptibility was not observed in a second TgFABP-PVR × TgPVR line produced from different founder mice (27).

Lack of poliovirus replication in the alimentary tract of TgFABP-PVR and TgFABP-PVR × TgPVR mice. To ascertain whether poliovirus can replicate in the alimentary tract of TgFABP-PVR mice, virus titers in the feces were determined at different times after oral inoculation. Small amounts of virus, presumably representing a portion of the inoculum, were observed at days 0.5 and 1.5 after infection of TgFABP-PVR × TgPVR and TgFABP-PVR mice; thereafter no virus was detected (Table 3). Similar results were obtained when homogenates of the entire small intestine were assayed for the presence of infectious virus (27). These results indicate that expression of high levels of Pvr in the mouse intestinal epithelium is not sufficient to confer susceptibility to poliovirus infection.

Poliovirus binding to TgFABP-PVR intestine. Lack of poliovirus susceptibility of the alimentary tract of TgFABP-PVR mice might result from failure of poliovirus to bind the intestinal epithelium. To address this possibility, pieces of the small intestine from normal and transgenic mice were prepared, and their ability to bind poliovirus was monitored by neutralization assay. Intestinal fragments were mixed with poliovirus and incubated at room temperature for 2 h. After centrifugation, poliovirus infectivity in the supernatant was determined by plaque assay on HeLa cell monolayers. Titers of poliovirus were significantly reduced when intestinal fragments from the duodenum or jejunum of TgFABP-PVR mice were used (Fig. 3). Incubation of poliovirus with intestinal fragments from either nontransgenic or TgPVR mice only slightly reduced infectivity. These results indicate that Pvr expressed on the cell surface of the intestinal epithelium is capable of binding poliovirus. Therefore, the absence of poliovirus infection in the gut of TgFABP-PVR mice is not due to the inability of Pvr to bind virus.

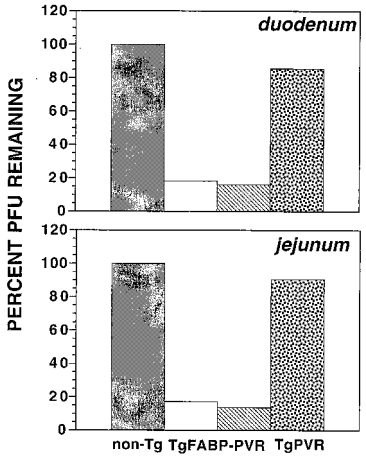

FIG. 3. Poliovirus binding activity in mouse small intestine. Pieces of small intestine from normal and transgenic mice were prepared and incubated with poliovirus at room temperature, and the supernatant was assayed for poliovirus infectivity. The virus input used was 5×10^6 PFU; 3.5×10^6 PFU remained after incubation with nontransgenic mouse intestine. Two different TgFABP-PVR lines were used (two middle bars).

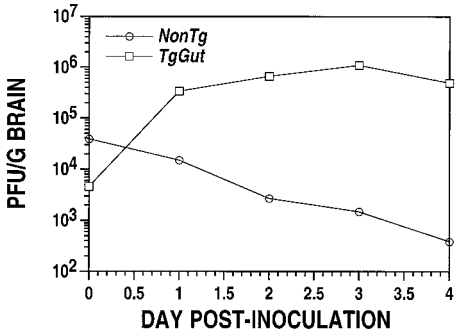

FIG. 4. Poliovirus replication in mouse brain. Ten nontransgenic mice (NonTg) and ten TgFABP-PVR mice (TgGut) were inoculated intracerebrally with 10^5 PFU of poliovirus. Two mice from each group were sacrificed daily, and virus titer in the brain was determined by plaque assay of homogenates on HeLa cell monolayers. Each time point represents the average of values obtained from two mice.

Poliovirus replication in the brain of TgFABP-PVR mice. To show that Pvr expressed by the transgene in TgFABP-PVR mice is functional, transgenic and normal mice were inoculated intracerebrally with poliovirus, and virus replication in the brain was assayed at different times postinfection. Poliovirus replicated in TgFABP-PVR mouse brain but not in the brain of normal mice (Fig. 4). Virus titers in TgFABP-PVR mouse brain peaked at 4 days postinoculation, consistent with the results of experiments carried out in TgPVR mice (18). TgFABP-PVR mice also developed paralytic poliomyelitis after intracerebral inoculation (Table 2). The rat I-FABP promoter has been reported to function aberrantly in tissues other than the intestine (28), which most likely accounts for the susceptibility of TgFABP-PVR mouse brain to poliovirus infection. Mice homozygous for the FABP-PVR transgene appear to be more susceptible to intracerebral inoculation than heterozygous animals (Table 2). The basis for this difference is not known.

DISCUSSION

An orally susceptible mouse model for poliovirus infection has certain advantages over currently available transgenic mouse models. Oral infection is the natural route of poliomyelitis transmission in the human population (reviewed in reference 17). Several steps in the pathogenesis of poliomyelitis, including the initial site of virus replication in the intestine and the mechanism by which poliovirus spreads from the gut to the central nervous system, are still not well characterized. An orally susceptible mouse could be used to study the intestinal immune responses to poliovirus infection and might be valuable for testing new vaccines.

To address the hypothesis that the absence of poliovirus replication in the TgPVR mouse alimentary tract is due to the low levels of PVR expression in the small intestine (17, 19), we established new transgenic mouse lines by using PVR cDNA under the control of the rat I-FABP promoter. TgFABP-PVR mice were not susceptible to oral inoculation with poliovirus, although Pvr was expressed in enterocytes and M cells and was able to bind poliovirus. These results indicate that the inability of poliovirus to replicate in mouse alimentary tract of TgPVR mice is not solely due to low levels of virus receptor.

There are several possible explanations for the failure of poliovirus to replicate in the intestine of TgFABP-PVR mice. If the Peyer's patch, not the enterocyte, is the primary site of poliovirus replication, then TgFABP-PVR mice would not be susceptible to infection because the rat I-FABP promoter does not function in lymphocytes. Alternatively, Pvr expressed in the transgenic mouse intestine might not be posttranslationally modified in a manner that permits productive entry of virus into cells. Another possibility is that mouse enterocytes lack a functional host factor required for poliovirus entry. Due to the difficulties of assaying the uncoating of poliovirus in mouse enterocytes, we were unable to determine whether poliovirus RNA can enter these cells. An isoform of CD44 has been implicated in poliovirus attachment (23, 24), but recent data indicate that CD44 is not required for poliovirus infection (2, 7). Other host factors likely to be required for postentry events in virus replication, including translation, genome replication, and capsid assembly, might be absent or sufficiently different in murine enterocytes to preclude virus replication.

Our finding that expression of Pvr in the mouse intestine is not sufficient to overcome a block to poliovirus replication is consistent with Sabin's theory that animals at a lower position on the evolutionary ladder are less susceptible to oral poliovirus inoculation (21). According to this theory, which is supported by experimental observations, the alimentary tract of monkeys is resistant to poliovirus infection, while that of chimpanzees is susceptible, and the gut of humans is the most susceptible. Based on this hierarchy, the intestine of mice should be highly resistant to poliovirus infection, which is supported by the results presented here. Interestingly, the susceptibility of the CNS of primates is the inverse of the susceptibilities of their intestinal tracts (21). Consistent with this hierarchical ranking, the CNS of PVR transgenic mice is susceptible to poliovirus infection (11, 18).

We have occasionally detected poliovirus in the feces of transgenic mice well after the initial levels of excreted virus have diminished (27). Experiments are currently under way to determine whether viruses isolated after day 4 postinoculation are mutants that can overcome the block to replication in the mouse intestine. The isolation and study of poliovirus mutants that can multiply in the intestine of TgFABP-PVR mice would provide information about the mechanism of resistance of the mouse gut to poliovirus infection.

ACKNOWLEDGMENTS

We thank Frank Costantini and Xiaolin Liang for instruction on the production of transgenic founders, Jeffrey Gordon for the rat I-FABP promoter, and Gail McMullen for instruction on the oral inoculation of mice.

This work was supported by a grant from Wyeth-Lederle Vaccines and Pediatrics.

REFERENCES

1. **Bodian, D.** 1955. Emerging concept of poliomyelitis infection. Science **12:** 105–108.
2. **Bouchard, M., and V. R. Racaniello.** 1997. CD44 is not required for poliovirus replication. J. Virol. **71:**2793–2798.
3. **Brinster, R. L., J. M. Allen, R. R. Behringer, R. E. Gelinas, and R. D. Palmiter.** 1988. Introns increase transcriptional efficiency in transgenic mice. Proc. Natl. Acad. Sci. USA **85:**836–840.
4. **Brown, R. H., D. Johnson, M. Ogonowski, and H. L. Weiner.** 1987. Type 1 human poliovirus binds to human synaptosomes. Ann. Neurol. **21:**64–70.
5. **Cormack, D. H.** 1987. Ham's histology, 9th ed. J. B. Lippincott Co., Philadelphia, Pa.
6. **Freistadt, M. F., G. Kaplan, and V. R. Racaniello.** 1990. Heterogeneous expression of poliovirus receptor-related proteins in human cells and tissues. Mol. Cell. Biol. **10:**5700–5706.
7. **Freistadt, M. S., and K. E. Eberle.** 1996. CD44 is not required for poliovirus replication in cultured cells and does not limit replication in monocytes. Virology **224:**542–547.
8. **Hogan, B., F. Costantini, and E. Lacy.** 1986. Manipulating the mouse embryo: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Holland, J. J.** 1961. Receptor affinities as major determinants of enterovirus tissue tropisms in humans. Virology **15:**312–326.

10. **Kanamitsu, M., A. Kasamaki, M. Ogawa, S. Kasahara, and M. Imamura.** 1967. Immunofluorescent study on the pathogenesis of oral infection of poliovirus in monkey. Jpn. J. Med. Sci. Biol. **20:**175–191.
11. **Koike, S., C. Taya, T. Kurata, S. Abe, I. Ise, H. Yonekawa, and A. Nomoto.** 1991. Transgenic mice susceptible to poliovirus. Proc. Natl. Acad. Sci. USA **88:**951–955.
12. **Lang, K. M., and R. A. Spritz.** 1985. Cloning specific complete polyadenylated 3′-terminal cDNA segments. Gene **33:**191–196.
13. **Mendelsohn, C., E. Wimmer, and V. R. Racaniello.** 1989. Cellular receptor for poliovirus: molecular cloning, nucleotide sequence and expression of a new member of the immunoglobulin superfamily. Cell **56:**855–865.
14. **Morrison, M. E., Y.-J. He, M. W. Wien, J. M. Hogle, and V. R. Racaniello.** 1994. Homolog-scanning mutagenesis reveals poliovirus residues important for virus binding and replication. J. Virol. **68:**2578–2588.
15. **Racaniello, V. R.** 1995. Early events in infection: receptor binding and cell entry, p. 73–93. *In* H. A. Rotbart (ed.), Human enterovirus infection. ASM Press, Washington, D.C.
16. **Racaniello, V. R., and D. Baltimore.** 1981. Cloned poliovirus complementary DNA is infectious in mammalian cells. Science **214:**916–919.
17. **Racaniello, V. R., and R. Ren.** 1996. Poliovirus biology and pathogenesis. Curr. Top. Microbiol. Immunol. **206:**305–325.
18. **Ren, R., F. C. Costantini, E. J. Gorgacz, J. J. Lee, and V. R. Racaniello.** 1990. Transgenic mice expressing a human poliovirus receptor: a new model for poliomyelitis. Cell **63:**353–362.
19. **Ren, R., and V. R. Racaniello.** 1991. Unpublished data.
20. **Rueckert, R. R.** 1990. Picornaviruses and their replication, p. 507–548. *In* B. N. Fields, D. M. Knipe, R. M. Chanock, M. S. Hirsch, J. L. Melnick, T. P. Monath, and B. Roizman (ed.), Virology. Raven Press, New York, N.Y.
21. **Sabin, A. B.** 1956. Pathogenesis of poliomyelitis: reappraisal in light of new data. Science **123:**1151–1157.
22. **Sabin, A. B., and R. Ward.** 1941. The natural history of human poliomyelitis. I. Distribution of virus in nervous and non-nervous tissues. J. Exp. Med. **73:**771–793.
23. **Shepley, M., and V. Racaniello.** 1994. A monoclonal antibody that blocks poliovirus attachment recognizes the lymphocyte homing receptor CD44. J. Virol. **68:**1301–1308.
24. **Shepley, M. P., B. Sherry, and H. L. Weiner.** 1988. Monoclonal antibody identification of a 100-kDa membrane protein in HeLa cells and human spinal cord involved in poliovirus attachment. Proc. Natl. Acad. Sci. USA **85:**7743–7747.
25. **Sweetser, D. A., E. H. Birkenmeier, I. J. Klisak, S. Zollman, R. S. Sparkes, T. Mohandas, A. J. Lusis, and J. I. Gordon.** 1987. The human and rodent intestinal fatty acid binding protein genes. J. Biol. Chem. **262:**16060–16071.
26. **Sweetser, D. A., S. M. Hauft, P. C. Hoppe, E. H. Birkenmeier, and J. I. Gordon.** 1988. Transgenic mice containing intestinal fatty acid-binding protein-human growth hormone fusion genes exhibit correct regional and cell-specific expression of the reporter gene in their small intestine. Proc. Natl. Acad. Sci. USA **85:**9611–9615.
27. **Zhang, S., and V. R. Racaniello.** 1996. Unpublished data.
28. **Zhou, L., C. R. Dey, S. E. Wert, J. A. Whitsett, M. D. DuVall, and R. A. Frizell.** 1994. Correction of lethal intestinal defect in a mouse model of cystic fibrosis. Science **266:**1705–1708.