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Molecular Cloning and Expression of a Murine Homolog of the Human Poliovirus Receptor Gene

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The poliovirus receptor (Pvr) is a member of the immunoglobulin superfamily of proteins, but its function in the cell is not known. Southern blot hybridization analysis indicated that the murine genome contains a sequence homolog of *pvr*. As a first step toward using the murine *pvr* homolog (*mph*) to study the function of Pvr, murine genomic and cDNA clones encoding *mph* were isolated. *mph* encodes a polypeptide with extensive sequence similarity to the extracellular domains of the human PVR. *mph* mRNAs of 2.0 and 3.0 kb are transcribed in the adult mouse brain, the spinal cord, the spleen, the kidney, the heart, and the liver. The Mph protein does not function as a receptor for poliovirus. However, substitution of domain 1 of the Mph protein with the corresponding sequence from *pvr* produced a chimeric receptor that could bind poliovirus and lead to productive infection. By constructing *pvr-mph* chimeras, it will be possible to identify the contact points of poliovirus within domain 1 of Pvr. Identification of the ligand and the cellular function of the Mph protein may help us understand the role of Pvr in the cell.

Recently, several cell proteins have been identified as receptors for different viruses. In some cases, these proteins have known cell functions; for example, the human immunodeficiency virus type 1 (HIV-1) receptor, CD4, and the Epstein-Barr virus receptor, CR2, participate in cell recognition, while the major group rhinovirus receptor, ICAM-1, is an adhesion molecule (5, 12, 15, 28, 29). However, while the cell receptor for poliovirus has been identified (14), its natural function remains unknown. The poliovirus receptor (Pvr) is an integral membrane protein that is a member of the immunoglobulin superfamily of proteins (14). The Pvr protein contains one V-like and two C-like extracellular immunoglobulin domains and therefore may act as an adhesion or cell surface recognition molecule.

Information on the function of Pvr might be obtained from the study of a murine Pvr homolog (Mph). For example, the distribution of putative Mph ligands would be more easily determined with mice. Homologous recombination experiments to create an *mph* null allelle in mice might enable elucidation of its function. Finally, the ligand- and virus-binding sites of the human Pvr could be explored by creating recombinants between *pvr* and *mph*.

To facilitate research into the natural function of the Pvr, we have isolated genomic and cDNA clones for a murine sequence homolog. *mph* has a high degree of amino acid and nucleotide similarity to *pvr* as well as a conserved splicing pattern. *mph* transcripts are detected in all adult mouse tissues tested. Mph is not a receptor for any of the three serotypes of poliovirus; however, substituting the first domain of the Mph with that of the Pvr enables the recombinant protein to serve as a Pvr. The amino acid residues crucial to interaction with poliovirus therefore reside in domain 1.

MATERIALS AND METHODS

Cells and viruses. 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's modified Eagle's medium (DMEM) containing 10% horse serum and gentamicin. Mouse L cells were maintained in DMEM containing 10% calf serum, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 20 μg of gentamicin per ml. Transient DNA transformants were grown in DMEM with 10% fetal bovine serum, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 20 μg of gentamicin per ml. Stable DNA transformants were grown in the same medium with 100 mM hypoxanthine–0.4 mM aminopterin–16 mM thymidine (HAT). Poliovirus strains P1/Mahoney and P2/Lansing were derived from the infectious cDNA clones (17, 18).

DNA transformation. ltk^- $aprt^-$ cells were seeded in plastic cell culture plates 1 day before use (2×10^6 cells per 6-cm-diameter plate for transient assay; 7.5×10^5 cells per 10-cm-diameter plate for isolation of stable transformants). The medium was changed 4 to 6 h before transformation. Plates were treated with the following DNA-calcium phosphate coprecipitates: for transient assay, 0.5 ml of a mixture of 10 μg of plasmid DNA and 10 μg of herring sperm DNA; for stable transformation, 1.0 ml of a mixture of 10 μg of plasmid DNA and 3 μg of a plasmid containing the herpesvirus thymidine kinase gene. After 18 h of incubation at 37°C, the medium was replaced and incubation was continued for 24 h. For transient assay, cells were infected with poliovirus 48 h after transformation. For isolation of stable transformants, cells were grown in HAT medium for 2 weeks and HAT-resistant colonies were subcultured.

Virus infection. Monolayers of L-cell transformants were infected with P1/Mahoney or P2/Lansing at a multiplicity of infection of 5, as indicated in the figure legends. After a 45-min adsorption period at 37°C, plates were washed with phosphate-buffered saline four times to remove unadsorbed virus, and the medium was replaced. Aliquots of supernatants were removed at different times after infection, and virus titers were determined by plaque assay on HeLa cell monolayers (10).

RNA and DNA isolation. Total RNA was isolated from cultured cells and tissues by homogenization in 4 M guani-

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dine isothiocyanate followed by centrifugation on a cushion of CsCl (2). Poly(A)$^+$ RNA was purified by chromatography on oligo(dT)-cellulose columns according to the manufacturer's instructions (Collaborative Research). Genomic DNA was prepared from cultured cells as described previously (13). Mouse genomic DNA was prepared from tail samples as described previously (6).

Northern (RNA) and Southern hybridization. RNA was fractionated on 1% agarose-formaldehyde gels and transferred to Zeta-Probe membranes (Bio-Rad) according to the manufacturer's instructions. High-stringency blots were prehybridized for 4 h at 42°C in a solution containing 50% formamide, 5× SSCPE (1× SSCPE is 0.15 M NaCl, 0.015 M sodium citrate, 13 mM KH_2PO_4, and 1 mM EDTA [pH 7.2]), 10× Denhardt's solution, 1% sodium dodecyl sulfate (SDS), and 500 μg of boiled, sheared herring sperm DNA per ml and hybridized for 18 h at 42°C in a solution containing 50% formamide, 5× SSCPE, 2× Denhardt's solution, 1% SDS, 100 μg of herring sperm DNA per ml, and 5 ng of DNA probe per ml. High-stringency blots were washed three times for 5 min each at room temperature and then once for 30 min at 65°C in 1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–1% SDS. Low-stringency Northern blots were hybridized in the same solution but were incubated at 37°C. Low-stringency blots were washed three times at room temperature and once at 55°C in 2× SSC–1% SDS. For Southern analysis, genomic DNA was digested with 4 U of restriction endonucleases per microgram overnight and fractionated on 0.8% agarose gels. DNA was transferred to nitrocellulose filters as described previously (27). High-stringency hybridization conditions were as described previously (Schleicher & Schuell). Low-stringency Southern blots were prehybridized for 4 h at 42°C in a solution containing 1 M NaCl, 50 mM Tris-HCl (pH 7.4), 35% formamide, 0.5% SDS, 5× Denhardt's solution, and 1 mg of herring sperm DNA per ml and hybridized for 18 h at 42°C in a solution containing 1 M NaCl, 50 mM Tris-HCl (pH 7.4), 35% formamide, 0.5% SDS, 1× Denhardt's solution, 10% dextran sulfate, 1 mg of herring sperm DNA per ml, and 5 ng of DNA probe per ml. Low-stringency blots were washed three times at room temperature and once for 30 min at 55°C in 2× SSC–0.1% SDS. ^{32}P-labelled probes were prepared by oligonucleotide-primed synthesis (Pharmacia).

cDNA, genomic, and cosmid libraries. cDNA was synthesized as described previously (14) from twice-oligo(dT)-selected mRNAs from the brains of Swiss Webster mice or mouse L cells. Double-stranded, *Eco*RI-adapted cDNA was ligated to *Eco*RI-digested λZAP arms (Stratagene) and packaged by using Gigapack Gold extracts (Stratagene). A C57/BL genomic DNA library in EMBL3 was kindly provided by Glenn Radice. Unamplified cDNA or amplified genomic libraries of 10^6 recombinants were plated on *Escherichia coli* LE392 cells. Duplicate filters were hybridized with DNA probes under the conditions described above for Southern blots. λZAP autoexcision of insert-containing pBluescript was carried out according to Stratagene's instructions. A genomic cosmid library from C57 mice was obtained from the laboratory of Fred Alt, and duplicate filters were screened as described previously (20).

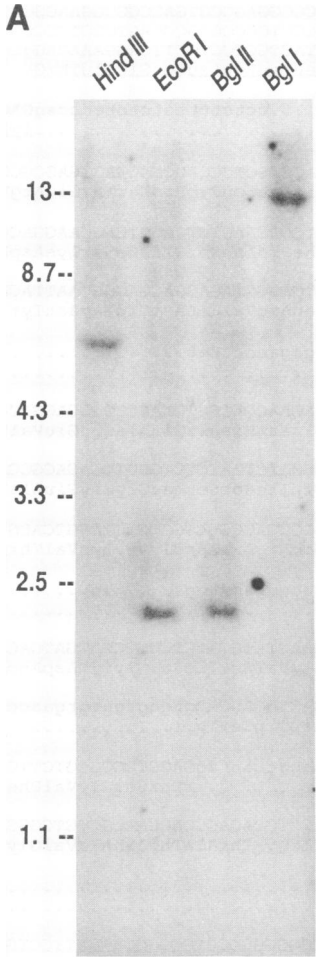
DNA sequencing and sequence comparisons. Subcloned restriction fragments from cDNA clones were sequenced by the dideoxy method (23) either manually or by automated sequencing (ABI, Inc.). Sequences were compiled and analyzed with the University of Wisconsin Genetics Computer Group sequence analysis package (4). Alignments of the human PVR sequence and the murine homolog sequence were made by using the Genetics Computer Group program BESTFIT, with default settings.

5′ end reconstruction. Polymerase chain reaction (PCR) was used to reconstruct the 5′ end of the longest brain-derived cDNA clone. Briefly, genomic sequencing of cosmid 2 DNA provided the correct 5′ 26-nucleotide sequence. A 5′ PCR primer, bATG2, containing an *Eco*RI site, the missing 26-nucleotides, and a 25-bp overlap with brain cDNA clone 2 and a 3′ PCR primer, bTGA, overlapping the termination codon and also containing an *Eco*RI site were used to amplify the coding region of brain cDNA clone 2: bATG2, 5′CTGGAATTCCCCATGGCCCGGGCCGCAGTCCTCC CGCCGTCCAGATTGTCACCGACGCTG3′; bTGA, 3′CG TCAAATGCACACTGGGATGCTTAAGGTC5′. PCR was carried out for 30 cycles at 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min. The product was cloned into M13 vectors for sequence confirmation and into other vectors for expression studies as described below. The reconstructed cDNA in M13 is designated pMEM.brmp18.

Expression of cDNA clones. The PCR-reconstructed murine brain cDNA was cleaved with *Eco*RI, the ends were made blunt with Klenow enzyme (BMB), and the resulting fragment was cloned into an *Sma*I-digested expression vector, pSVL (Pharmacia). This plasmid is designated pMEM. brSVL7. Some recombinants with the human *pvr* cDNA were constructed as described below. To determine the Pvr activity of these cDNAs, L cells were transformed with CsCl-purified plasmids as described above. Transformants were assayed for their sensitivity to poliovirus infection and stable cell lines were isolated as described above.

Oligonucleotide-directed mutagenesis. Oligonucleotide-directed mutagenesis was performed on human *pvr* cDNAs subcloned in M13 and grown in *E. coli* CJ236 as described previously (1). The enzymes used were T4 polynucleotide kinase (BMB) and Klenow enzyme (BMB). Nucleotide changes were introduced into human receptor cDNA inserts corresponding to plasmids pSVL-H20A and pSVL-H20B (14) to create a *Kpn*I site between immunoglobulinlike domains 1 and 2 in a position analogous to this *Kpn*I site in the murine brain cDNA sequence. These mutations result in a coding change from serine to threonine at amino acid 132. The M13 human *pvr* clones with the new *Kpn*I site are designated pMEM.20AKpn and pMEM.20BKpn. The mutagenized coding regions were cloned back into expression vector pSVL as described previously (14). These constructs are designated pMEM.20AKpnSVL and pMEM.20BK pnSVL.

Construction of recombinants. Recombinants between the human *pvr* cDNA clone in pMEM.20BKpn and the reconstructed murine brain cDNA clone in pMEM.brmp18 were constructed. Briefly, plasmid pMEM.brmp18 was digested with *Sma*I and *Kpn*I and treated with calf intestine alkaline phosphatase; the 373-bp fragment containing murine domain 2 and the 7,872-bp fragment containing the M13mp18 vector with *mph* domain 3 through the cytoplasmic tail were isolated from low-melting-point agarose gels. Plasmid pMEM.20BKpn was digested with *Sma*I and *Kpn*I, and the 507-bp fragment containing *pvr* domain 1 and the 373-bp fragment containing *pvr* domain 2 were isolated from low-melting-point agarose gels. The 507-bp *pvr* domain 1 *Sma*I-*Kpn*I fragment was ligated into the 7,872-bp *mph* domain 3-cytoplasmic tail-M13mp18 fragment, creating plasmid pMEM.1. In this process, the *Sma*I and *Bam*HI sites from the M13 vector were deleted, but the *Xba*I site was left intact. The domain 2 sequence was restored to pMEM.1 by linearizing this plasmid with *Kpn*I and ligating in the 373-bp



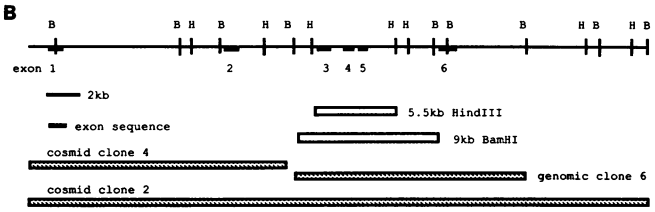


FIG. 1. Murine DNA contains sequences homologous to those of the human *pvr*. (A) Genomic DNA from C57BL mouse spleen was digested with the indicated restriction enzymes and analyzed by Southern hybridization, at low stringency, with a 0.97-kb *Eco*RI human *pvr* cDNA probe encoding the 3′ half of domain 1 and all of extracellular domains 2 and 3. The positions of molecular weight markers (in kilobases) are indicated on the left. (B) Restriction map and exon distribution of the *mph* gene. A combination of low-stringency hybridization with human *pvr* cDNAs and high-stringency hybridization with *mph* cDNA was used to isolate and map the genomic DNA region shown. Solid bars indicate exon sequences; numbers below these bars correspond to the exon numbers in Fig. 2. Open bars indicate the restriction fragments observed by low-stringency Southern hybridization with human *pvr* probes. Stippled bars indicate the extent of individual genomic and cosmid clones. B, *Bam*HI; H, *Hin*dIII.

domain 2 *Kpn*I fragment, creating plasmids pMEM.h5 and pMEM.m1. The chimeric junctions were confirmed by restriction endonuclease digestion and DNA sequence analysis. The chimeric inserts were excised by total *Xba*I and partial *Eco*RI digestion, the termini were made blunt with Klenow enzyme, and the inserts were cloned into *Sma*I-digested pSVL vector. The final constructs are as follows: pMEM.m1-1 contains PVR domain 1 up to the *Kpn*I site introduced by mutagenesis, followed by murine sequences 3′ of the *Kpn*I site, and pMEM.h5-1 contains *pvr* domains 1 and 2 up to the naturally occurring *Kpn*I site between domains 2 and 3, followed by murine sequences for domain 3, the putative transmembrane-spanning region, and the cytoplasmic tail. pMEM.h5-1 contains the *Kpn*I site introduced by mutagenesis of the human receptor cDNA.

Nucleotide sequence accession number. The GenBank accession number for the *mph* cDNA sequence is M12197.

RESULTS

Isolation of genomic and cDNA clones encoding Mph. To determine whether the mouse genome contains a sequence homolog of the human Pvr, mouse DNA was subjected to low-stringency Southern blot analysis with a 0.97-kb *Eco*RI DNA fragment probe from human *pvr* cDNA (14). This hybridization probe detected one DNA species, suggesting the presence of a single homologous gene (Fig. 1A). The human *pvr* cDNA probe was then used to screen a C57BL mouse genomic library, and one type of recombinant phage represented by genomic clone 6 was isolated (Fig. 1B). This genomic clone contained the 5.5-kb *Hin*dIII and the 2.1-kb *Eco*RI restriction fragments detected by genomic Southern analysis in Fig. 1A. Genomic clone 6 also contained a 9-kb *Bam*HI fragment, which was detected by Southern analysis of mouse DNA (16).

A 0.6-kb *Bgl*II-*Eco*RI fragment was isolated from genomic clone 6 and used to screen several cDNA libraries. The most complete cDNA clone was isolated from a mouse brain library and is designated pMEM.b2. The nucleotide sequence of this clone is discussed below. The entire 1.4-kb coding region of pMEM.b2 was used to screen a cosmid library of C57BL mouse DNA. Two classes of cosmid clones were isolated; the bars below the genomic DNA map in Fig. 1B indicate the location of DNAs from cosmid clones 2 and 4. These clones both hybridized with probes from the 5′ end of brain cDNA clone 2, but only cosmid 2 also hybridized with a 3′ end probe (16). Both classes of cosmid clones contain DNA fragments found in genomic clone 6, as indicated by restriction mapping.

Nucleotide sequence analysis of *mph* cDNA clones. Nucleotide sequence analysis of *mph* cDNA and genomic clones revealed the intron-exon structure and the coding regions shown in Fig. 2. pMEM.b2 contains an open reading frame of 1,377 bp which encodes a 459-amino-acid polypeptide composed of a 24-residue amino-terminal hydrophobic signal sequence, a 314-amino-acid extracellular domain, a 28-amino-acid transmembrane domain, and a 93-amino-acid cytoplasmic tail. Amino acid sequence alignment with the human Pvr indicates conservation of the three extracellular immunoglobulinlike domains across the species. The three domains (defined by intron-exon boundaries) share 52, 57, and 64% identity and 64, 68, and 80% similarity at the amino acid level (Fig. 3). The positions of the splice sites in the extracellular domains are conserved between the human and murine genes. Nonconsensus bases within the splice sites lie in the same positions, further emphasizing the homology between the *pvr* and the *mph* genes. The possible sites for N-linked glycosylation, indicated in Fig. 3, differ between

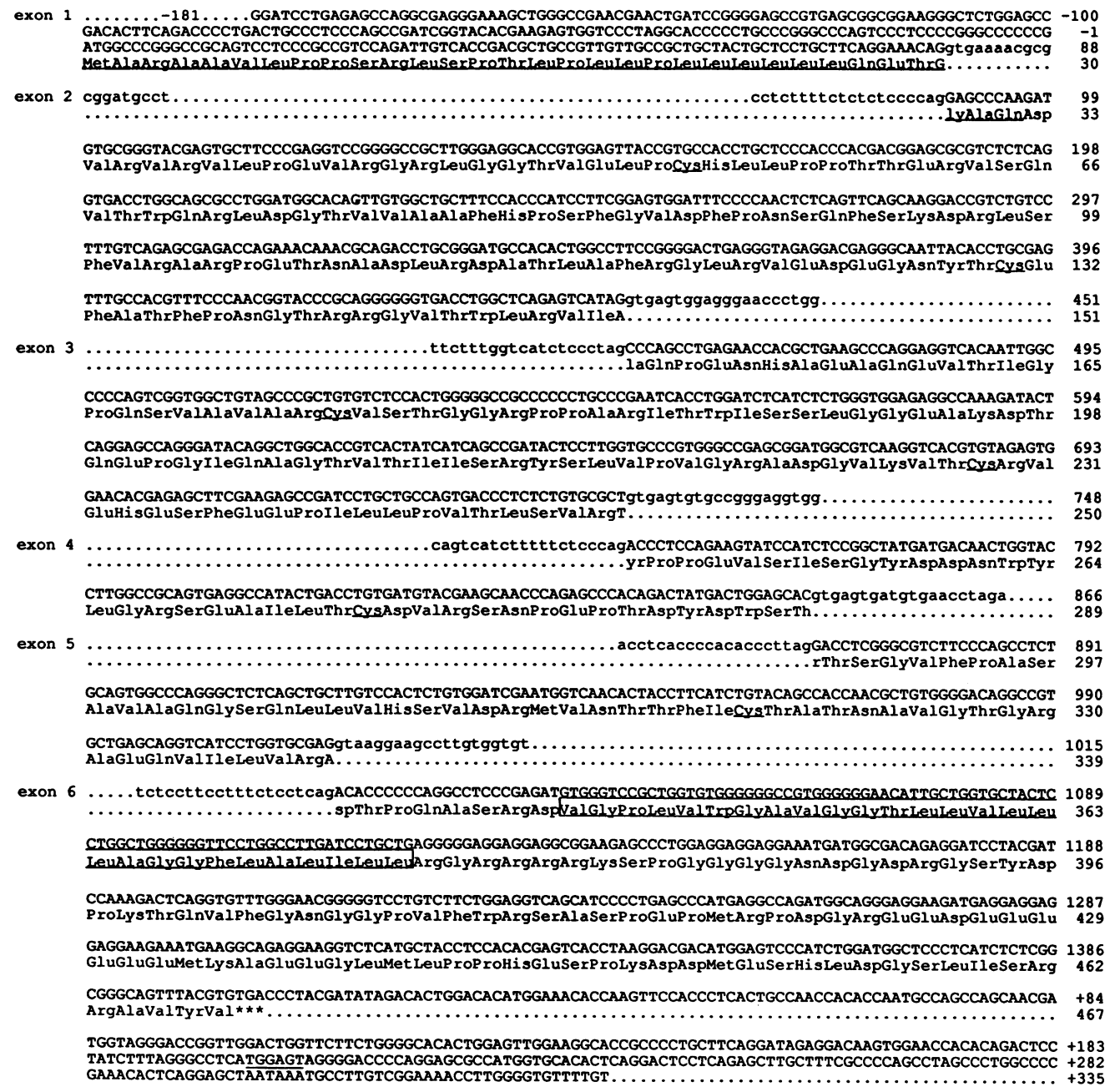


FIG. 2. Intron-exon structure of the *mph* 2.0-kb transcript. Capital letters represent exon nucleotide sequences; lowercase letters indicate intron sequences. Nucleotide positions relative to the coding region are shown at the right. Amino acid sequences are given below the nucleotide sequences, and amino acid positions are shown at the right. The putative signal peptide is underlined, and the putative transmembrane domain is boxed. Cysteines important to immunoglobulin family domain structure are also underlined, and the poly(A) signal is indicated by a line above the nucleotide sequence AATAAA.

the two cDNAs: the Pvr protein contains eight potential sites, whereas Mph contains only three. The transmembrane and cytoplasmic domains share little or no significant similarity.

Although pMEM.b2 contained an extensive coding region, it lacked an AUG translation initiation codon. Nucleotide sequencing of subcloned cosmid DNAs indicated that the missing AUG lay 23 bp upstream from the 5′-terminal nucleotide of brain clone 2, on the same exon as the 65 bp of signal sequence included in pMEM.b2 cDNA.

In vivo expression of *mph* mRNAs. Northern blot hybridization of RNA from adult mouse tissues was used to determine the distribution of *mph* transcripts. A murine cDNA probe containing sequences encoding extracellular domains 2 and 3, the transmembrane region, and the NH_2-terminal half of the cytoplasmic tail hybridized with 2- and 3-kb mRNAs in all tissues tested, including the brain, the spinal cord, the heart, the liver, the kidney, and the spleen (Fig. 4). Northern analysis using 3′-noncoding-region hybridization probes indicated that all the cDNA clones iso-

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    1 MARA..MAAAW...PLLLVALLVLSWPPPGTGDVVVQAPTQVPGFLGDSV 45   Pvr
      ||||  ::::.:     .| |:::||:| :...|..|| ...  .:|.| ||:.|
    1 MARAAVLPPSRLSPTLPLLPLLLLLLQETGAQDVRVPVLIEVRGRLGGTV 50   Mph

   46 TLPCYLQVPNMEVTHVSQLTWARHGESGSMAVFHQT.QGPSYSESK....R 91   Pvr
      .|||.| .|. |   :||||:||.|  ::.  :  .  ..  |..:.:|.    |
   51 ELPCHLLPPTTE..RVSQVTWQRL.DGTVVAAFHHRSFPVIFPNSQFSKDR 97   Mph

   92 LEFVAAR..LGAELRNASLRMFGLRVEDEGNYTCLFVTFPQGSRSVDIWL 139  Pvr
      |.|| ||     .:|:||:|.| :  :||||.|.|     .:|||.|.|.. .||
   98 LSFVRARPETNADLRDATLAFRGLRVEDEGNYTCEFATFPNGTRRGVTWL 147  Mph

  140 RVLAKPQNTAEVQKVQLTGEPVPMARCVSTGGRPPAQITWHSDLGGMPNT 189  Pvr
      ||:|.|:| ||.|.| :..:.|::|||||||||||||.||| |.||| :..
  148 RVIAQPENHAEAQEVTIGPQSVAVARCVSTGGRPPARITWISSLGGEAKD 197  Mph

  190 SQVPGFLSGTVTVTSLWILVPSSQVDGKNVTCKVEHESFEKPQLLTVNLT 239  Pvr
      .| ||: .|||||:.| : ||| :..|| .|||:|||||||.| ||.|.|.
  198 TQEPGIQAGTVTIISRYSLVPVGRADGVKVTCRVEHESFEEPILLPVTLS 247  Mph

  240 VYYPPEVSISGYDNNWYLGQNEATLTCDARSNPEPTGYNWSTTMGPLPPF 289  Pvr
      | ||||||||||||:|||||..||.||||.|||||||:|:||||| |.:.:
  248 VRYPPEVSISGYDDNWYLGRSEAILTCDVRSNPEPTDYDWSTTSGVFPAS 297  Mph

  290 AVAQGAQLLIRPVDKPINTTLICNVTNALGARQAELTVQVKEGPPSEH.. 337  Pvr
      |||||.|||::.||: :||||:||..|||:|. .|| .: |::.|...:
  298 AVAQGSQLLVHSVDRMVNTTFICTATNAVGTGRAEQVILVRDTPQASRDV 347  Mph

  338 .....SGISRNAIIFLVLGILVFLILL....................... 359  Pvr
           :::: . :::|: | :: ||||
  348 GPLVWGAVGGTLLVLLLAGGFLALILLRGRRRRKSPGGGNDGDRGSYDP 397  Mph

  360 .....GIGIYFYWSKCSREVLWHCHLCPSSEHHQSCRN 392........      Pvr
           | |  .:|...|.|.::.    ...|..:. :.
  398 KTQVFGNGGPVFWRSASPEPMRPDGREEDEEEEEEMKA 435........      Mph
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FIG. 3. Amino acid sequence comparison of Mph and Pvr. The University of Wisconsin Genetics Computer Group program BESTFIT was used to align the Mph and Pvr amino acid sequences. Identical and conserved amino acids are indicated by vertical lines and dots, respectively. The Pvr sequence is above and the Mph sequence is below the identity indicators. Potential N-linked glycosylation sites are underlined.

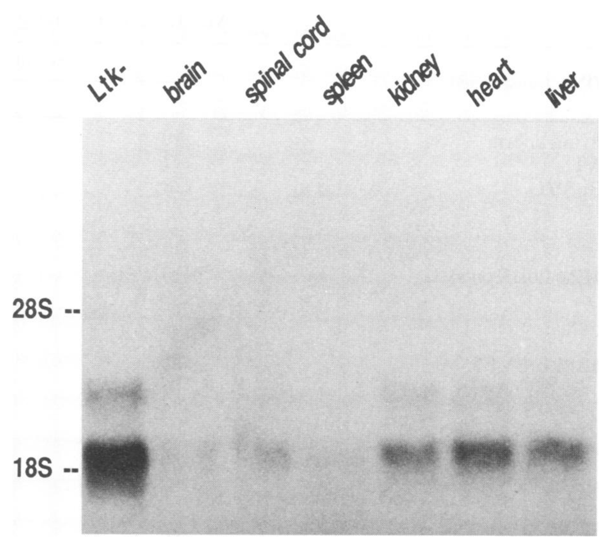

FIG. 4. Northern blot hybridization analysis of *mph* transcripts. Poly(A)$^+$ mRNAs from Swiss Webster mouse organs were electrophoresed in formaldehyde-agarose gels and subjected to Northern hybridization analysis with an *mph* probe encoding extracellular domains 2 and 3, the transmembrane region, and the amino-terminal half of the cytoplasmic tail (bp 463 to 1182 on the nucleotide sequence in Fig. 2). The positions of rRNAs are indicated at the left.

lated in these experiments are derived from the 2.0-kb mRNA (16). The 3.0-kb mRNA hybridized with pMEM.b2 probes from domain 1, domain 2, or domain 3 but did not hybridize with cytoplasmic tail or 3′ noncoding sequences from the same cDNA clone. These results indicate that the 2- and 3-kb mRNAs differ in the 3′ end. Both *mph* transcripts are present in L cells, a murine fibroblast line that is resistant to infection with all serotypes of poliovirus (Fig. 4).

Pvr activity of the Mph. The presence of *mph* transcripts in mouse L cells, which do not express poliovirus-binding sites, suggested that the Mph protein does not serve as a Pvr. To confirm this assumption, *mph* cDNA was assayed for Pvr activity. Because brain cDNA clone pMEM.b2 lacked the first 23 nucleotides of the polypeptide, the correct 5′ end for the coding region was reconstructed by using PCR, as described in Materials and Methods. The reconstructed coding region was sequenced in its entirety and cloned into the eukaryotic expression vector pSVL. This construct, designated pMEM.brSVL7, was transformed into L cells. At 48 h after transformation, the cells were infected with poliovirus P2/Lansing, a mouse-adapted strain, and 48 h later the supernatants were assayed for infectious poliovirus by plaque assay on HeLa cells. As shown in Table 1, expression of *mph* in L cells did not permit poliovirus infection of these cells, whereas control plasmids containing *pvr* cDNA conferred susceptibility to infection. Furthermore, virus-binding assays with stable cell lines expressing *mph* indicate that P2/Lansing does not bind to the Mph (16).

pvr-mph **recombinants.** It was of interest to construct *pvr-mph* chimeric molecules to identify the regions of the Mph that prevent its function as a Pvr. To facilitate the construction of these recombinants, *pvr* cDNA H20B was mutagenized to create a *Kpn*I site at the same location as a *Kpn*I site in *mph* cDNA between domains 1 and 2. This alteration results in a change from serine to threonine at human Pvr amino acid 132. This coding change has no effect on the mutagenized cDNA's ability to serve as a Pvr, as shown in Table 1; transformation of plasmids 20BpSVL and pMEM.20BKpnSVL into L cells lead to susceptibility to poliovirus infection. In this assay system, twofold differences are not significant.

The *mph-pvr* recombinants that were constructed are shown schematically in Table 1. Plasmid pMEM.m1-1 encodes a molecule with human Pvr domain 1 in place of the corresponding Mph domain, and plasmid pMEM.h5-1 encodes a molecule with human Pvr domains 1 and 2 in place of the Mph domains. As shown in Table 1, both of these plasmids confer sensitivity to poliovirus infection on L cells. Therefore, the amino acid differences in domain 1 of the Mph and the Pvr prevent the Mph from functioning as a Pvr. Since the Pvr and the Mph share extensive homology in domain 1, it should be possible, by substitution of murine Mph for Pvr residues in the individual loops and β strands of the first immunoglobulinlike domain, to identify the Pvr residues critical for poliovirus binding and infection.

DISCUSSION

Murine genomic and cDNA clones which encode a polypeptide, the Mph, with structural similarities to the human Pvr were isolated. Both the Pvr and the Mph include a signal peptide, three extracellular immunoglobulinlike domains, a transmembrane domain, and a cytoplasmic tail. High amino acid and nucleotide sequence conservation in the extracellular domains (64 to 80%) indicate a close relationship between *pvr* and *mph*; this level of conservation is comparable to that between human and murine ICAM-1 (7, 26) or human and murine CD4 (11), receptors for major group human rhinoviruses and HIV, respectively, and also members of the immunoglobulin superfamily of proteins. Chromosome mapping indicates that *mph* is located in a region of

TABLE 1. Pvr activity test of *mph* and *mph-pvr* chimeras[a]

DNA transformed	PFU/ml at (h): 0	PFU/ml at (h): 48	Receptor structure[b]
Herring sperm	46	5.2×10^4	
pSVL	212	4.6×10^4	
20BpSVL	128	2.9×10^7	
pMEM.20BKpnSVL	210	6.0×10^7	
pMEM.brSVL7	20	4.1×10^4	
pMEM.m1-1	142	6.0×10^7	
pMEM.h5-1	150	3.5×10^7	

[a] The plasmids indicated at the left were transiently transfected into L cells as described in Materials and Methods. Two days posttransformation, the cells were infected with P2/Lansing virus at a multiplicity of infection of 5. The inoculum was washed off, supernatant aliquots were removed at 0 and 48 h, and virus titers were determined by plaque assay on HeLa cell monolayers.

[b] The expected protein structures, with stippled domains representing Pvr sequences and open domains representing Mph sequences.

mouse chromosome 7 (8) which is known to be syntenic with human chromosome 19, to which the human *pvr* has been mapped (9, 24, 25). One significant difference between *mph* and *pvr* is the lack of alternative splicing of the 2.0-kb *mph* transcript. Examination of *mph* mRNAs by PCR showed that there is no form of the 2.0-kb message lacking a transmembrane domain (16), as is found for the Pvr (9).

The natural functions of both the Mph and the Pvr are unknown. It is possible that this new member of the immunoglobulin gene family is involved in cell adhesion or surface recognition, as are many other immunoglobulinlike proteins, including the rhinovirus receptor, ICAM-1 (5, 28, 29); the HIV receptor, CD4 (12); and the Epstein-Barr virus receptor, CR2 (15). Adhesion assays are being conducted to test this hypothesis. We have also begun homologous recombination experiments to explore the effects of a null allelle of the *mph* gene during mouse development. These studies of the *mph* gene may provide clues about its function. Whether or not the Mph protein is a functional homolog of the Pvr also remains to be determined.

The ubiquitous expression of *mph* in mouse organs, which is also observed with *pvr* in human tissues, does not rule out the possibility of highly regulated cell-specific expression within those tissues. Examination of *pvr* expression in transgenic mice by in situ hybridization indicates that the transgene is expressed only in specific cell types, including, for example, neurons within the central and peripheral nervous systems, glomeruli and tubular epithelial cells in the kidney, endocrine cells in the cortex of the adrenal gland, T lymphocytes in the cortex of the thymus, and alveolar macrophages (21). Whether or not a similar pattern of expression of *pvr* occurs in humans remains to be determined. Also of great interest is whether the pattern of expression of *mph* in mice is similar to those of *pvr* in transgenic mice and humans. In situ hybridization using *mph* probes is planned to define the patterns of *mph* expression within adult mouse tissues and during prenatal development.

Most strains of poliovirus cannot infect mice. This species restriction is due to the lack of receptors in mice, as demonstrated previously by the observation that expression of *pvr* in transgenic mice leads to susceptibility to poliovirus infection (20). However, some strains of poliovirus, such as the P2/Lansing strain, have been adapted to grow in nontransgenic mice. It is believed that these strains recognize a receptor in mice that cannot be recognized by strains that do not infect mice. A logical candidate for the mouse P2/Lansing receptor is *mph*. However, despite the close relationship between *pvr* and *mph* and the fact that a closer sequence homolog of *pvr* does not seem to exist in mice, our results indicate that *mph* cDNA encoding the 2-kb brain mRNA does not act as a Pvr when expressed in L cells. One possible explanation for this finding is that *mph* cDNA transformed into L cells does not result in the expression of a cell surface protein. We cannot rule out this possibility, because antibodies that recognize the Mph are not yet available. However, we believe it is unlikely that the Mph fails to reach the surfaces of transformed cells, because a protein in which domain 1 of the Mph is substituted with the corresponding sequence from the Pvr is clearly expressed on the cell surface. It is possible that L cells lack the modification machinery necessary for Mph receptor activity. Another possibility is that the 3-kb *mph* mRNA encodes a receptor for P2/Lansing. Molecular clones of this mRNA have not yet been obtained, and therefore direct tests of this hypothesis are not possible. However, because the 3-kb mRNA hybridizes with all extracellular portions of the 2-kb cDNA and, in addition, the extracellular domain 1 of the Pvr is sufficient to confer susceptibility to poliovirus infection, we believe it is unlikely that the 3-kb mRNA encodes a Lansing receptor.

The most likely explanation for the lack of Pvr function of the Mph is that mouse-adapted strains of poliovirus use a different molecule for their receptor. Multiple-receptor usage by a single virus has been observed with other viruses. For example, the coxsackievirus B3 variant CB3-RD was selected for growth in RD cells but maintained its ability to grow in HeLa cells (19). The finding that CB3 saturation of HeLa cell receptors failed to block CB3-RD binding indi-

cated a second receptor present on HeLa cells which the parent virus failed to recognize (3). Unfortunately, the molecular identity of these receptors is not yet known. Thus, it is possible that P2/Lansing uses a mouse receptor that is not the murine sequence homolog of the human *pvr*.

The close sequence homology of Mph and Pvr should enable identification of Pvr residues important for virus binding by substitution of Mph amino acids into the Pvr and vice versa. The results presented here demonstrate that domain 1 of the Pvr is sufficient to confer full Pvr activity on the Mph protein. It is now a matter of mutagenesis and cloning to define the individual loops and β strands of Pvr domain 1 that are important for virus binding, as was done for HIV-1 interaction with CD4 (22). It will be of interest to determine whether other parts of Pvr are required for subsequent steps in the poliovirus life cycle, such as the conformational transitions of the virion that are associated with virus entry into the cell, including the loss of the internal virion protein VP4, externalization of the VP1 NH_2 terminus, and extrusion of RNA from the capsid. CD4 sequences that are important for binding of HIV-1 are different from sequences that permit gp120-mediated membrane fusion (22). By analogy, different parts of Pvr may participate in binding and triggering conformational transitions, and Pvr mutants that separate those functions may provide a dynamic picture of virus-receptor contact and communication at the molecular level.

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