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Heterogeneous Expression of Poliovirus Receptor-Related Proteins in Human Cells and Tissues

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Portions of the cellular receptor for poliovirus were expressed in *Escherichia coli* as fusion proteins with the product of the *trpE* gene. One of two antireceptor antisera obtained by immunizing rabbits with the fusion proteins blocked poliovirus infection. Western immunoblot analyses demonstrated that poliovirus receptor-related proteins were expressed in HeLa cells and a variety of human tissues, including those that are not sites of poliovirus replication. Tissue-specific variation in electrophoretic mobility, immunoreactivity, and subunit arrangement of poliovirus receptor-related proteins was observed. These results demonstrate that poliovirus tissue tropism cannot be explained by a limited distribution of receptor polypeptide, but may be the result of alternative splicing, posttranslational modifications, or both. In addition, the widespread but heterogeneous expression of the receptor suggests that the protein may have an important endogenous function.

Cellular receptors for several viruses, including Epstein-Barr virus (EBV) (5, 14), human immunodeficiency virus type 1 (11), major group human rhinoviruses (HRVs) (6, 18), poliovirus (12), and murine ecotropic retrovirus (1), have recently been identified. These structurally divergent molecules appear to play different roles in viral tissue tropism. For example, the tissue distribution of the EBV receptor, complement receptor type 2 (CR2), is probably important in determining the extent of EBV infection. In contrast, intercellular adhesion molecule 1 (ICAM-1), the cellular receptor for the major group HRVs, is expressed in a wide range of tissues, yet viral replication is limited to the nasopharynx. Therefore, the role of ICAM-1 in restricting HRV replication is unclear. However, despite their divergent roles in viral life cycles, some viral receptors, such as ICAM-1 and CR2, are certainly essential host molecules. Other molecules which were initially identified as viral receptors (1, 12), although currently of unknown function, are also likely to be important to the host.

Poliovirus, the causative agent of paralytic poliomyelitis, replicates in a narrow range of human cell and tissue types, including oropharyngeal and intestinal mucosa, tonsils, Peyer's patches, cervical and mesenteric lymph nodes, and certain parts of the central nervous system (precentral gyrus [motor cortex] and specific areas of the cerebellum, brainstem, and spinal cord) (2). The molecular basis for this restricted tissue tropism is unknown. Although the results of binding studies suggest that poliovirus binding sites are found only in susceptible tissues, binding activity has also been reported in several tissues and cell types that are not sites of poliovirus replication (4, 7, 10).

The recent molecular cloning of cDNAs encoding functional cell receptors for poliovirus should allow an examination of the relationship between poliovirus tissue tropism and receptor expression (12). Two cDNAs have been molecularly cloned which direct the synthesis of a functional poliovirus receptor (PVR) after transformation into receptor-negative mouse cells. The predicted polypeptides encoded by the cDNAs are novel members of the immunoglobulin superfamily and consist of a putative leader peptide, three extracellular immunoglobulinlike domains, a transmembrane domain, and a short cytoplasmic domain. The two cDNAs encode proteins that differ only at the cytoplasmic tails. Northern (RNA blot) analysis of human tissue RNA revealed receptor-specific transcripts, of similar sizes, in both poliovirus-susceptible and nonsusceptible tissues (12). Therefore, poliovirus tissue tropism is not controlled by organ-specific transcriptional control of receptor mRNA.

Although a cellular receptor for poliovirus has been identified, nothing is known about the protein's structure and expression in vivo or about its natural cellular function. To begin to address these questions, we have isolated polyclonal antireceptor antisera. While monoclonal antibodies which block poliovirus infection have been isolated (13, 15, 17), this report describes the first generation and characterization of antibodies which can biochemically detect the receptor protein encoded by a functionally active cDNA (8, 12). The antisera detect a series of related polypeptides in HeLa cells and human tissues, including those that do not express poliovirus binding sites. The results suggest that tissue-specific alternative splicing, posttranslational modification, or both may modify the structure of this receptor, possibly mediating poliovirus tissue tropism. These modifications may affect the cellular function of the receptor.

MATERIALS AND METHODS

Viruses, cells, and other materials. Type 1 (Mahoney) poliovirus and HeLa S3 cells were used as described before (12). The *trpE* fusion vector pATH2 was kindly provided by T. J. Koerner and A. Tzagoloff. Restriction enzymes were purchased from New England BioLabs. Monoclonal antibody D171 (15) was obtained from P. Nobis. D171 and anti-TrpE-3D^{Pol} (9) were purified by affinity chromatography to final protein concentrations of 1 and 0.8 mg/ml, respectively. Protein molecular weight standards, stained with Coomassie dye, were purchased from Bethesda Research Laboratories.

Induction of fusion proteins. Single colonies of *Escherichia coli* harboring the pATH2 vector or fusion plasmids were used to inoculate overnight cultures of L broth supplemented with tryptophan to 20 μg/ml. The following day, the

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overnight cultures were diluted 100-fold into M9 minimal medium with no tryptophan. After 1 h at 30°C, indoleacrylic acid (Sigma) was added to 5 μg/ml (stock solution, 1 mg/ml in 100% ethanol). Cultures were harvested after 16 h of incubation at 30°C. Time course experiments showed that induction plateaued after 5 h and that the fusion proteins were stable during the overnight incubation (data not shown).

The induced bacteria were pelleted and suspended in a small volume (1/100 of the volume of medium) of cracking buffer (0.01 M $NaPO_4$ [pH 7.2], 1% β-mercaptoethanol, 1% sodium dodecyl sulfate [SDS], 6 M urea). The lysate was incubated at 37°C for 2 h, sonicated, and either frozen at −20°C or loaded onto polyacrylamide gels immediately.

Immunization. Induced proteins were visualized either by Coomassie staining (for analytical purposes) or in 0.25 M KCl–1 mM (dithioerythritol (DTE) for preparative gels, and specific bands were excised. For the primary injections, gel slices were manually macerated finely enough to flow through a 27.5-gauge needle and then mixed with an equal volume of 50% (vol/vol) Freund complete adjuvant (Sigma) in sterile phosphate-buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4). Approximately 200 μg of protein was injected into female New Zealand White outbred rabbits in 0.2-ml injections (10 per rabbit). Boosts were administered every 3 to 4 weeks, followed by bleeding 7 to 10 days later. For boosts, 20 to 50 μg of protein was eluted from chopped gel slices by incubation in 0.05 M Tris (pH 7.9)–0.1 mM EDTA–5 mM DTE–0.1% SDS overnight at 37°C. The proteins were then filtered, mixed with an equal volume of 50% (vol/vol) Freund incomplete adjuvant (Sigma) in sterile PBS, and injected in several 0.2-ml portions.

Viral infection blocking assay. HeLa cells (5×10^5) were seeded into the wells of a 24-well plate. When the cells were confluent, the medium was aspirated, the cells were washed two times in Dulbecco modified essential medium (DMEM; GIBCO) with 2% fetal calf serum (FCS), and the antibody or antiserum, diluted into DMEM with 2% FCS, was added to the specified concentration. All incubations were done at 37°C in a CO_2 incubator. The cells were incubated with the antibodies for 2 h; then virus was added and allowed to adsorb for 45 min at 37°C. Medium was added, and the cells were observed for cytopathic effects. Cells were stained with crystal violet as described before (16).

The ability of the antisera to block poliovirus replication was quantitated in a plaque reduction assay. Confluent HeLa cells (2×10^6) in 6-cm plates were washed in DMEM with 2% FCS and incubated for 2 h with various concentrations of antiserum or antibodies. Approximately 100 PFU of type 1 Mahoney poliovirus was adsorbed to cells for 45 min at 37°C. DMEM with 1% agar (Difco) was then added, plaques were allowed to develop for 48 h, and the cells were stained with crystal violet as described before (16).

Western immunoblot analysis. Cytoplasmic extracts from insect cells infected with baculoviruses were prepared as described before (8). Normal human tissues from adult autopsies were homogenized in lysis buffer (1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride [Sigma], 5 mM EDTA, 0.5 mg of aprotinin [Sigma] per ml, 0.1 mM E64 [Calbiochem], 0.1 mg of pepstatin [Calbiochem] per ml in PBS) in a Brinkmann homogenizer. The lysate was transferred to Eppendorf tubes, and nuclei and unlysed cells were immediately removed by centrifugation. HeLa cells (grown in spinner flasks) were centrifuged, suspended in lysis buffer, and incubated on ice for 10 min, and the nuclei were pelleted. For the human cells and tissues, 0.1% SDS was added to the low-speed supernatant; then it was centrifuged for 1 h at 100,000 × *g* at 4°C. The protein content of the high-speed supernatant was determined by the Bradford assay (3). Samples were added to an equal volume of gel loading buffer (with or without DTE), boiled for 4 min, and electrophoresed through polyacrylamide gels.

Proteins were transferred from the gels to nitrocellulose filters with a Transphor electrophoretic transfer unit (Hoefer) in 0.192 M glycine–25 mM Tris–0.1% SDS–20% methanol for 2 h at 200 mA at room temperature with stirring. The filters were rinsed two times for 5 min each in rinse buffer (0.2% Tween-20 [Sigma] in TBS [0.05 M Tris chloride (pH 7.4), 0.15 M NaCl]). The incubations and rinses were carried out with gentle rotation at room temperature. Excess sites were blocked by incubating for 40 min in rinse buffer containing 5% fish gelatin (Sigma G-7765), 1% Nonidet P-40, and 0.1% SDS. The filters were rinsed two times in rinse buffer and then incubated in the primary antiserum diluted into binding buffer (rinse buffer with 1% fish gelatin, 1% Nonidet P-40, and 0.1% SDS) for 40 min. The blots were washed five times for 5 min each in the rinse buffer to remove unbound antibodies. The secondary antibody (peroxidase- or alkaline phosphatase-conjugated to anti-rabbit immunoglobulin G [IgG; Sigma]) was diluted into binding buffer and applied to the filters for 40 min. The filters were rinsed five times for 5 min each. For alkaline phosphatase-conjugated secondary antibody, the filters were rinsed two times in 0.1 M Tris chloride (pH 9.5)–0.1 M NaCl–5 mM $MgCl_2$ at room temperature. The color reaction was developed in 0.01% Nitro Blue Tetrazolium and 0.01% indoxyl phosphate (both from Sigma) in the previous buffer. For peroxidase-conjugated secondary antibodies, the filters were developed in 0.05 M sodium acetate, pH 5.0, containing a 1/300 dilution each of 30% H_2O_2 and 3-amino-9-ethyl-carbazole (Sigma)-saturated ethanol.

Serum absorption. Crude lysates from bacteria (prepared as described above from about 10 ml of saturated culture) harboring fusion proteins or from baculovirus-infected insect cells (about 10^7 cells prepared as described in reference 8) were used to absorb sera. Proteins to be used for absorption were diluted 1/10 into TBS and bound to a small square of nitrocellulose for 1 h at room temperature in a petri dish. The filter was rinsed four times in Western blot rinse buffer. From 3 to 4 ml of the serum to be absorbed was then added to the filter, which was then incubated for 1 h at room temperature with gentle rotation. After the unbound serum was removed, bound antibodies were eluted in a small volume of 0.05 M glycine (pH 2.5)–0.15 M NaCl for 20 min at room temperature, neutralized with 1 M Tris, pH 7.5 (usually about 5 volumes), and dialyzed against TBS. Bovine serum albumin (to 1 mg/ml) and sodium azide (to 0.05%) were added for storage at 4°C.

RESULTS

Generation of antisera. Two fusion proteins, each containing 38 kDa of anthranilate synthetase (the *trpE* gene product) and part of PVR, were generated by using pATH vectors. The sequence of the PVR protein predicts three immunoglobulinlike domains (12). One fusion protein, called 1i, contained almost the entire coding region, from the middle of the first domain to a *Hin*dIII site beyond the 3′ end of the coding region (Fig. 1). The second fusion construct, called 4a, contained only domain 1 with the putative leader sequence. Bacteria containing the fusion plasmids were induced, and

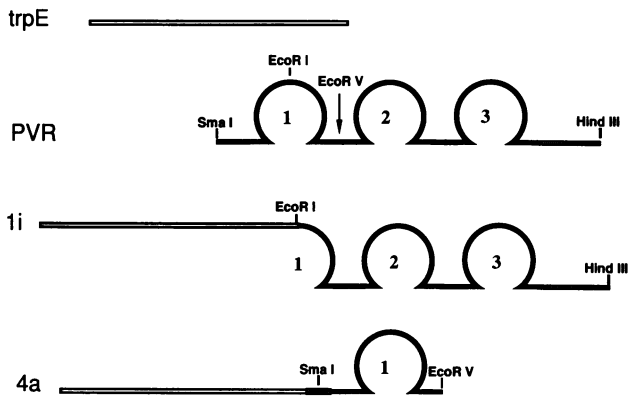

FIG. 1. TrpE-poliovirus receptor fusion proteins used to generate antisera. The bacterial protein TrpE (open box) was fused in frame to portions of the PVR cDNA (PVR; solid and hatched boxes). The hatched regions represent (from left to right) the putative signal peptide and transmembrane domain. Immunoglobulinlike domains 1, 2, and 3 are numbered from the N-terminus, or membrane-distal end. Fusion 1i contains sequences from the middle of domain 1 to a *Hind*III site beyond the 3′ end of the coding region. Fusion 4a contains primarily sequences from domain 1.

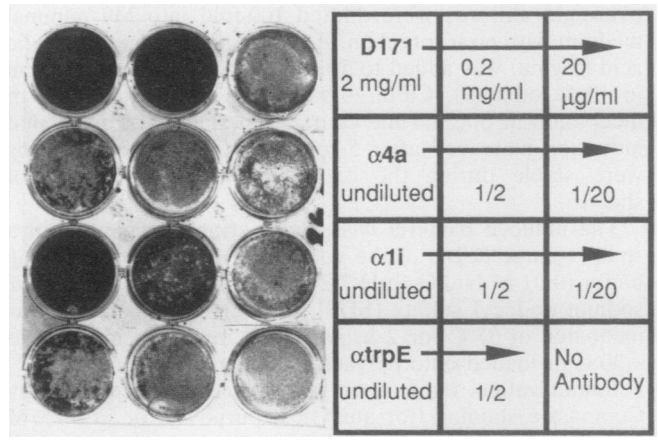

FIG. 2. Anti-1i protects HeLa cells from poliovirus infection. Confluent HeLa cells were incubated with the indicated antibody or antiserum at the specified concentrations for 2 h at 37°C. The cells were then challenged with poliovirus at a multiplicity of infection of 0.5. Cells were observed for cytopathic effects and stained with crystal violet, which stains living cells, 26 h after infection. Uninfected cells or a protecting antibody (such as D171 and anti-1i [α1i]) yielded stained cells (which appear dark), while antisera which do not protect (such as anti-4a [α4a] and anti-TrpE [αtrpE]) resulted in a cleared area. A control well without antibody is also shown.

proteins were analyzed by SDS-polyacrylamide gel electrophoresis. Extracts from bacteria harboring 1i or 4a contained fusion proteins migrating at the predicted molecular masses of 66 and 45 kDa, respectively (data not shown). Rabbits were immunized and boosted with preparations of 1i, 4a, and TrpE proteins. The resulting sera were screened regularly until a positive response against the surface of HeLa cells was obtained (data not shown). While anti-TrpE reactivity was detected within 6 weeks, no positive response was obtained against the PVR until 6 months after the primary injection.

Characterization of antisera. Two anti-fusion protein antisera which reacted with the surface of HeLa cells were selected for further study. The specificity of these sera for PVR was tested by assaying their ability to block poliovirus infection in a cell protection assay (Fig. 2) and in a plaque reduction assay (Table 1). Confluent HeLa cell monolayers were incubated with various dilutions of the antisera for 2 h at 37°C. The cells were then challenged with poliovirus, and the extent of reduction of cytophathic effect was observed by staining with crystal violet, which stains living cells. An intact, stained monolayer indicates protection by the antibody, while failure to protect results in detachment of infected cells and clearing of areas on the plate. Monoclonal antibody was isolated by virtue of its ability to protect HeLa cells from infection by poliovirus (15). As expected, D171 protected cells from poliovirus infection (Fig. 2). Anti-1i antiserum protected cells from poliovirus infection, while anti-4a and anti-TrpE did not (Fig. 2). Cell protection was quantitated by a plaque reduction assay (Table 1). D171 and anti-1i reduced poliovirus plaque formation by 88 and 56%, respectively, while anti-4a, anti-TrpE, and anti-TrpE-3D^{pol} (antiserum directed against a TrpE-poliovirus RNA polymerase fusion protein) did not protect against infection.

Additional evidence that the antisera contain anti-PVR antibodies was obtained from Western blot analysis of extracts from insect cells infected with a baculovirus which expresses the PVR (8). Cytoplasmic extracts of insect cells infected with wild-type baculovirus did not react with normal rabbit serum, anti-1i, or anti-4a (Fig. 3). In contrast, anti-1i and anti-4a, but not normal rabbit serum, reacted with a diffuse polypeptide of 55 to 68 kDa in extracts of insect cells infected with a recombinant baculovirus containing PVR cDNA (Fig. 3).

Western blot analysis of proteins from human cells and tissues. To characterize the expression of PVR proteins in HeLa cells and human tissues, detergent extracts from various sources were examined. Autopsy specimens were obtained from tissues known to support poliovirus replication (motor cortex, cerebellar cortex, brainstem, spinal cord, and ileum) and from tissues that are not sites of poliovirus replication (kidney and liver). HeLa cells and tissue samples were lysed in detergents and prepared for blotting as described in Materials and Methods. In HeLa cell protein, both anti-PVR antisera predominantly identified a diffuse series of polypeptides from 55 to 69 kDa. In addition, reactivity to proteins of about 80, 100, and 150 kDa was observed (Fig. 4). Other proteins of about 30 and 40 kDa were not consistently detected.

Each of the human tissues studied contained proteins of approximately similar molecular masses which reacted with both antisera. However, the pattern of reactivity differed

TABLE 1. Poliovirus plaque reduction assay

Antibody	% Inhibition[a]		
	Undil	1:2	1:50
Anti-TrpE	0	3	—
Anti-TrpE-3D^{pol}[b]	—	—	0
D171	—	—	88
Anti-4a	0	0	—
Anti-1i	56	41	—

[a] Percent inhibition was calculated by subtracting the ratio of the number of plaques after antibody incubation to the number of plaques after buffer incubation from 1 and multiplying by 100. The number of plaques after incubation with buffer was 110. Undil, Antiserum or antibody not diluted. —, Not done.

[b] Raised against a TrpE-3D^{pol} fusion protein (9).

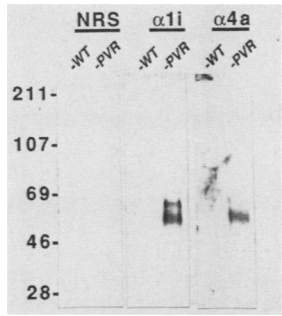

FIG. 3. Western blot analysis of baculovirus-expressed proteins. Insect cells infected with different baculoviruses were solubilized in 1% Nonidet P-40, and nuclei were pelleted (8). A 750-ng amount of protein from each cytoplasmic extract was electrophoresed in three identical 10% polyacrylamide gels, transferred to nitrocellulose sheets, incubated with a 1:100 dilution of unabsorbed normal rabbit serum (NRS) or the indicated rabbit immune serum, each previously absorbed to its cognate bacterially expressed TrpE fusion protein, and visualized after a second incubation with a 1:400 dilution of anti-rabbit IgG conjugated to alkaline phosphatase. In each panel, the left lane depicts protein from the insect cells infected with wild-type (WT) baculovirus, and the right lane shows protein from insect cells infected with the recombinant baculovirus expressing PVR. Relative molecular masses are indicated (in kilodaltons).

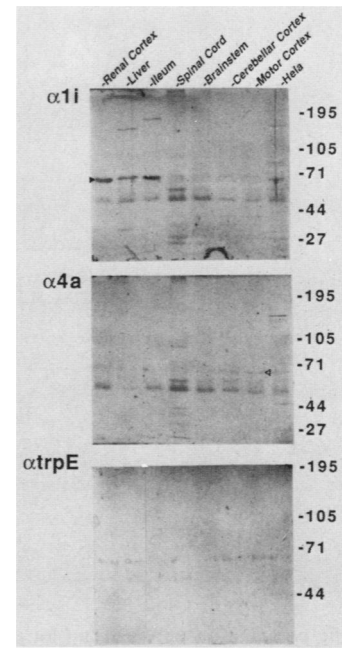

FIG. 4. Detection of poliovirus receptor proteins in Western blots of HeLa cell and human tissue proteins. High-speed supernatants from detergent-solubilized renal cortex (1.1 μg of protein), liver (10 μg), ileum (2 μg), spinal cord (32 μg), brainstem (36 μg), cerebellar cortex (30 μg), motor cortex (30 μg), and HeLa cell (40 μg) samples were electrophoresed in identical 10% polyacrylamide gels. Proteins from the gels were transferred to nitrocellulose filters, incubated with a 1:100 dilution of absorbed anti-1i (α1i), anti-4a (α4a), or anti-TrpE-3D^{pol} (αtrpE). The anti-PVR antisera were absorbed to the baculovirus-expressed PVR, while the anti-TrpE-poliovirus replicase had been purified with protein A (9). Reacting proteins were visualized after a second incubation with a 1:300 dilution of anti-rabbit IgG conjugated to peroxidase. Some of the proteins discussed in the text are indicated: the anti-1i-reactive 69-kDa renal cortex protein (solid arrowhead) and the anti-4a-reactive 68-kDa renal cortex protein (open arrowhead). Relative molecular masses are indicated (in kilodaltons).

between tissues. In renal cortex, ileum, and liver, anti-1i detected a protein of 69 kDa (solid arrowhead) and another, less intensely reacting protein of 55 kDa, while anti-4a reacted less strongly with the 69-kDa polypeptide. Spinal cord, brainstem, cerebellar cortex, and motor cortex tissues contained a more diffuse and heterogeneous series of proteins (sometimes displaying as many as five species) from 55 to 69 kDa that were reactive with both antisera. In these tissues, the 55-kDa protein was the predominant species detected by both sera. However, motor cortex contained a sharply defined protein of approximately 69 kDa which was more reactive with anti-4a than with anti-1i (open arrowhead). Other proteins which reacted with the anti-PVR antisera were proteins of 80 to 100 kDa, noted in the neural tissues; a sharp 150-kDa protein band, detectable with both antisera in liver tissue (although weak with anti-4a in this experiment); and an approximately 170-kDa protein in ileum, reactive with anti-1i only. Similar results were obtained with tissue specimens from several cadavers.

To determine the contribution of anti-TrpE antibodies to the signals observed in Western blots, an identical blot was probed with antiserum generated against a fusion polypeptide of TrpE with the poliovirus RNA polymerase (9). The anti-TrpE-3D^{pol} antiserum reacted weakly with polypeptides in some tissues, although these polypeptides migrated differently from those which reacted with the anti-PVR antisera (Fig. 4). Results of the experiment described below suggest that this weak reactivity was probably due to anti-poliovirus RNA polymerase antibodies and therefore probably does not account for the reactivities observed with anti-4a and anti-1i.

To further demonstrate that the proteins which reacted in Western blots with the anti-PVR antisera were related to the PVR, a competition experiment was carried out. Two gels, each containing five identical pairs of samples, were transferred to nitrocellulose. After transfer, the nitrocellulose was cut into strips and probed either with antiserum alone or with increasing amounts of competing protein. Proteins loaded on the gels were identical except that the strips probed with anti-1i contained renal cortex and HeLa cell protein, while strips probed with anti-4a contained motor cortex and HeLa cell protein. Extracts from either receptor-expressing or wild-type baculovirus-infected insect cells (8) were used as competitors. In each case, the signal from tissues or HeLa cells was fully competed out by the baculovirus-expressed receptor protein but not by extracts from wild-type baculovirus-infected insect cells (Fig. 5). These results demonstrate that all the polypeptides observed in Western blots with anti-1i or anti-4a are related to the PVR.

To examine whether PVR-related proteins form complexes in cells, the effect of reduction on polypeptide pattern was determined by Western blot analysis of samples with and without reduction by DTE. Most of the receptor-related proteins detected in renal cortex, cerebellar cortex, motor cortex, and HeLa cells migrated more slowly and diffusely when the sample was unreduced than when the sample was reduced (Fig. 6). However, when unreduced, one species in motor cortex (open arrowhead) migrated as a sharply defined polypeptide of approximately 69 kDa.

DISCUSSION

In this work, human tissues were examined for the presence of proteins related to the product of the PVR gene, although the proteins were not characterized for function as

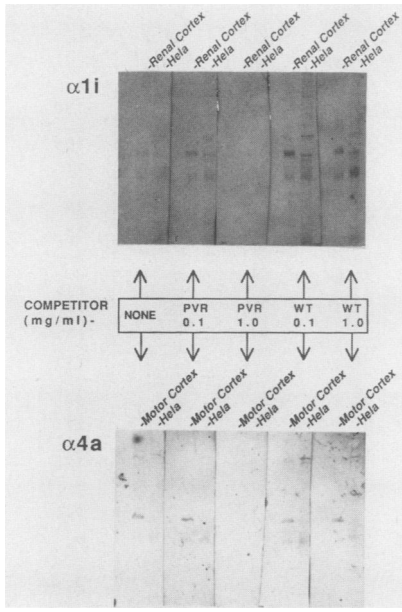

FIG. 5. Specific competition of Western blot immunoreactivity by baculovirus-expressed receptor. Two gels, each containing five identical pairs of samples (as indicated), were transferred to nitrocellulose. The amounts of protein loaded in each lane were: renal cortex, 0.5 μg; motor cortex, 29 μg; HeLa cell, 45 μg. After transfer, the nitrocellulose was cut into strips and probed with the indicated antiserum and competitor (protein from insect cells infected with wild-type baculovirus [WT] or recombinant baculovirus expressing PVR [PVR]) at the specified concentration. The antisera, previously absorbed to baculovirus-expressed receptor, were present in each incubation at a 1:50 dilution. Anti-rabbit IgG conjugated to peroxidase, the secondary antibody, was used at a 1:300 dilution.

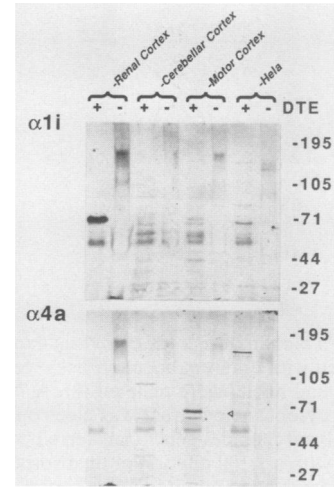

FIG. 6. Effect of treatment with DTE on PVR-related proteins. Lysates from renal cortex (1.1 μg), cerebellar cortex (36 μg), motor cortex (48 μg), and HeLa cell (75 μg of protein) samples were either treated (+) or not treated (−) with DTE prior to electrophoresis in 10% polyacrylamide gels. After transfer to nitrocellulose, the filters were probed with a 1:50 dilution of the indicated antiserum which had been absorbed to the baculovirus-expressed PVR, followed by a 1:300 dilution of anti-rabbit IgG conjugated to peroxidase. The protein discussed in the text is indicated with an open arrowhead. Relative molecular masses are indicated (in kilodaltons). Note that due to the loss of signal when the reducing agent was omitted, more protein was loaded onto these gels than in the previous experiments.

viral receptors. Two polyclonal antisera specific for the PVR were generated and characterized. Receptor-related protein was detected in cultured cells and in human tissues. We found, in contrast to the restricted viral tissue tropism, widespread expression in human tissues of PVR-related protein. Heterogeneity in molecular mass, immunoreactivity, and subunit arrangement was observed, suggesting that alternative splicing of the transcript, differential posttranslational modification of the protein, or both may occur. These modifications may be relevant to poliovirus tissue tropism as well as to the natural function of this protein.

The specificity of the antisera was demonstrated in several assays. One of the antisera, anti-1i, directed against nearly the entire receptor protein, inhibited poliovirus replication. Both antisera reacted with PVR expressed in insect cells. Finally, the reactivity of both antisera with human tissue and HeLa cell proteins in Western blots was fully competed out by extracts from insect cells infected with a PVR-expressing baculovirus and not by extracts from insect cells infected with wild-type baculovirus.

Anti-4a (against only domain 1 of the PVR) did not block poliovirus infection. There are several possible explanations for this result. Sequences in domain 1 may not be involved in virus binding and uptake. Alternatively, if the virus binding site is carbohydrate or a combination of peptide and carbohydrate, the anti-fusion polypeptide antiserum may not block virus-receptor interactions.

Two related PVR cDNAs, H20A and H20B, were previously cloned from HeLa cell mRNA (12). These two cDNAs encode polypeptides with predicted molecular masses of 45 and 43 kDa that differ only at their extreme C-termini. The antisera developed in this study do not distinguish between these two proteins. Species of the molecular masses predicted for the unmodified PVR polypeptides were not consistently observed, so it was not possible to distinguish between H20A and H20B polypeptides by electrophoretic mobility.

There are eight potential N-linked glycosylation sites in the predicted amino acid sequence of the PVR (12). The addition of eight carbohydrate groups of about 2,000 Da each would result in proteins of approximately 60 kDa. Consistent with utilization of most of the glycosylation sites, both antisera detected a diffuse doublet of 55 to 68 kDa in extracts from insect cells infected with the PVR-expressing baculovirus (Fig. 2). When insect cells expressing the PVR were treated with tunicamycin, the apparent molecular mass was reduced to 35 kDa (8), demonstrating that this protein is glycosylated. Whether glycosylation contributes to the patterns of PVR polypeptides observed in tissues remains to be determined.

Three kinds of tissue-specific heterogeneity in PVR-related proteins—molecular mass, immunoreactivity, and subunit arrangement—were detected by Western blot analysis. Although each tissue examined contained a series of PVR-related proteins from 55 to 69 kDa, the molecular mass of the predominant species reactive with each of the antisera differed between tissues (Fig. 4 and 6). This was most apparent with the anti-1i reactivity: in renal cortex, ileum, and liver, the 69-kDa species reacted more strongly than the 55-kDa species, while in the other tissues studied (spinal cord, brainstem, cerebellar cortex, and motor cortex) a protein of 55 kDa reacted more strongly (Fig. 4). This heterogeneity may be due to varying abundance of each species or to differential reactivity with the antisera.

Another type of variation of the receptor protein was differential reactivity between the two antisera. Anti-4a but not anti-1i reacted strongly with a sharply defined protein of approximately 69 kDa in motor cortex (Fig. 4 and 6), a poliovirus-susceptible tissue, but a similar protein was not seen in liver or ileum. In contrast, anti-1i but not anti-4a reacted strongly with a 69-kDa protein in ileum, renal cortex, and liver. Note that these antisera were generated against different bacterially produced polypeptide portions of the PVR. This differential reactivity suggests that these antisera detect tissue-specific variability in posttranslational modification.

A third type of tissue-specific difference was in susceptibility to reduction by DTE. Most of the PVR-reactive proteins displayed slower mobility in the absence of reduction, suggesting intermolecular disulfide linkages. However, one protein (likely the 69-kDa protein) in the motor cortex retained its sharpness, migrating similarly or slightly faster in the absence of reduction (Fig. 6, open arrowhead). This finding suggests that most forms of the PVR-related protein are either homo- or heteromultimeric species, while a motor cortex form of the protein might be monomeric. The PVR gene is a member of the immunoglobulin gene superfamily; the polypeptides predicted from the H20A and H20B cDNAs each contain six cysteines (in the putative extracellular domain) that may allow folding of the protein into three immunoglobulinlike domains, as well as additional cysteines in the putative cytoplasmic tail (12). An increase in electrophoretic mobility in the absence of reducing agents is characteristic of intramolecular disulfide bonding, as observed among members of the immunoglobulin superfamily (18).

A novel protein of approximately 150 kDa was detected in both HeLa cell and liver proteins. The 150-kDa species is probably not an unreduced form of smaller proteins, because unreduced, high-molecular-mass samples were very diffuse (Fig. 6), while this protein consistently migrated as a sharp band. It is possible that this protein is encoded by the 5.6-kb mRNA which was previously detected in both tissue culture and human tissues (12). Because the 5.6-kb mRNA was not molecularly cloned, it is not known whether it is a product of the PVR gene or a related gene. Whether the 150-kDa protein can function as a poliovirus receptor is not known, although it is unlikely to be active in vivo because it is not found in many tissues that express poliovirus binding sites. The source of other PVR-related proteins observed (e.g., 170 kDa in ileum, 100 kDa in spinal cord) is also not known.

Two 3.3-kb PVR-specific transcripts have been detected in many human tissues, including those that are not sites of poliovirus replication (12). The work described here indicates that PVR-related polypeptides are found in a similar range of human tissues. Therefore, translational control is not a likely mechanism for controlling expression of poliovirus binding sites, unless translation is restricted in a cell type-specific manner within tissues. Tissue-specific alternative splicing, glycosylation of PVR polypeptides, or both are possible mechanisms for controlling expression of poliovirus binding sites that are supported by the data presented here. The finding that the renal cortex but not the motor cortex form of the PVR is covalently bound either to itself or to another protein suggests that other proteins may also affect viral binding and uptake in vivo. Poliovirus may utilize subtle tissue-specific differences which are relevant to the normal cell function of the PVR. Finally, it is important to note that while functional receptors are required for infection of tissues, the presence of functional receptors does not ensure that replication will occur. Cells may contain blocks to poliovirus replication at stages other than virus binding and entry.

A monoclonal antibody has been isolated that blocks infection of HeLa cells with poliovirus types 1 and 2 (17). This antibody reacts with a 100-kDa polypeptide from HeLa cell membranes in Western blot analysis. The relationship between the proteins detected in the current work and the epitope detected by Shepley et al. (17) is unknown.

This work establishes a biochemical basis for determining the molecular mechanisms controlling poliovirus tissue tropism. Additional information on the modification and subcellular localization of PVR-related protein in human tissues, as well as its virus-binding activity, will be necessary to determine the role of the PVR in viral tissue tropism and should help to identify its natural function.

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