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Neutralization of Poliovirus by Cell Receptors Expressed in Insect Cells

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To examine the interaction of the poliovirus receptor (PVR) with virus and the role of the PVR in virus entry, the PVR was expressed in insect cells. Poliovirus bound to insect cells infected with a recombinant baculovirus (AcPVR) carrying cDNA encoding the PVR. Antibodies raised against PVR expressed in bacteria immunoprecipitated a 67-kilodalton polypeptide from cytoplasmic extracts of AcPVR-infected cells. Treatment of AcPVR-infected cells with tunicamycin revealed that the PVR is a glycoprotein containing N-glycosidic linkages and that carbohydrate accounts for nearly 50% of its molecular weight as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. When PVR was solubilized from AcPVR-infected insect cells and incubated with poliovirus, viral infectivity was neutralized. Sedimentation analysis revealed that irreversibly altered 135S particles were formed after incubation of poliovirus at 37°C with solubilized extracts of AcPVR-infected insect cells. These results demonstrate that poliovirus eclipse may result from interaction with the cell receptor at neutral pH in the absence of membranes and suggest that soluble receptors may be effective antiviral agents against picornaviruses.

Poliovirus, a small, nonenveloped animal virus, begins infection by binding to a cell receptor that is expressed only on certain primate cells and cell cultures (reviewed in reference 31). The identity of the poliovirus receptor (PVR) was unknown until recently, when cDNA clones encoding a functional human PVR were isolated (28). Expression of PVR cDNAs in mouse L cells permits infection of these normally resistant cells. These cDNA clones encode a novel polypeptide that is composed of three immunoglobulinlike domains, a transmembrane sequence, and a cytoplasmic tail. Two PVR cDNAs were isolated which encode polypeptides of 43 and 45 kilodaltons (kDa) that differ only in the length of the cytoplasmic tail. The putative polypeptides contain eight potential N-linked glycosylation sites, suggesting that the PVR is a glycoprotein.

Although examination of the polypeptides encoded by PVR cDNAs is informative, the actual constitution of the poliovirus-binding site is not known. To enable detailed analysis of the structure and function of the PVR, the polypeptide was expressed in insect cells. Immunoprecipitation analysis with anti-PVR antiserum demonstrated that the receptor was synthesized in insect cells as a heterogeneous glycoprotein, with a predominant species of 67 kDa. Poliovirus bound to insect cells expressing the PVR. A solubilized extract of insect cells expressing PVR neutralized virus infectivity by converting native virus to altered particles, in the absence of membranes or low pH.

MATERIALS AND METHODS

Cells, virus, and antisera. *Autographa californica* nuclear polyhedrosis virus (AcNPV) wild-type strain E1 and a recombinant containing PVR (AcPVR) were propagated in *Spodoptera frugiperda* IPLB-SF-21 cells as described previously (33). *S. frugiperda* cells were grown in either spinner cultures or monolayers with TMN-FH medium (33) containing 5% fetal bovine serum, 5% horse serum, 10 μg of gentamicin per ml, and 2.5 μg of amphotericin B per ml.

HeLa S3 cells were grown in Spinner cultures and plated in 6-cm plastic cell culture dishes (2×10^6 cells per dish) for plaque assays or 15-cm plastic cell culture dishes (6×10^7 cells per dish) for propagation of poliovirus P1/Mahoney (17).

Anti-PVR antiserum was obtained from rabbits immunized with a *trpE*-PVR fusion protein produced in *Escherichia coli* (M. Freistadt, G. Kaplan, and V. Racaniello, submitted for publication). The fusion polypeptide contained 38 kDa of the *trpE* gene product joined to all but the 93 N-terminal amino acids of the PVR. Monoclonal antibody D171 directed against the HeLa cell PVR (29) was purified and diluted to a concentration of 1 mg/ml. Anti-mouse polyvalent immunoglobulin-peroxidase conjugate was obtained from Sigma Chemical Co.

Construction and isolation of AcPVR cDNA. A 2,724-base-pair (bp) *Sal*I-*Sac*II DNA fragment from plasmid pSVL-H20B (28), which encodes a functional PVR, was made blunt ended with Klenow enzyme (25) and cloned into the filled-in *Bam*HI site of transplacement vector pAc373 (33). The *Sal*I-*Sac*II DNA fragment contains 32 bp from the 5′ noncoding region, the entire PVR-coding sequence, 1,007 bp from the 3′ noncoding region, and a 509-bp fragment from the pSVL vector including the simian virus 40 late polyadenylation sequences. The resulting plasmid, pAcPVR, contains the receptor cDNA under the control of the polyhedrin promoter. Genomic DNA extracted from purified wild-type baculovirus (AcNPV) was cotransfected with pAcPVR into *S. frugiperda* IPLB-SF-21 cells, and recombinant baculoviruses containing PVR cDNA (AcPVR) were purified by dilution and six rounds of plaque purification (33).

Enzyme-linked immunosorbent assay. Confluent monolayers of *S. frugiperda* cells were grown in 96-well plates and infected with either AcNPV or AcPVR at a multiplicity of infection (MOI) of 10. After 48 h, cells were treated with twofold dilutions of monoclonal antibody D171 and stained with anti-mouse polyvalent immunoglobulins-peroxidase

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conjugate and *o*-phenylenediamine dihydrochloride as described previously (16).

Poliovirus binding assay. Confluent *S. frugiperda* cell monolayers in 10-cm plates were infected with either AcNPV or AcPVR at an MOI of 10. After 48 h, medium was removed and 10^4 PFU of poliovirus P1/Mahoney in 0.3 ml of TMN-FH was added. The virus binding assay was performed as described previously (16). Samples of the cell culture medium were taken at 0, 1, and 2 h postinfection, and poliovirus titers were determined by plaque assay in HeLa cells. The percentage of adsorbed virus was calculated as the mean of three independent experiments.

Metabolic radiolabeling and immunoprecipitation. Confluent *S. frugiperda* cell monolayers in 10-cm dishes were infected with either AcNPV or AcPVR at an MOI of 10 and incubated at 28°C for 48 h. Monolayers were washed with TC-100 medium lacking methionine and incubated with the same medium for 2 h; then medium was removed and 6 ml of TC-100 lacking methionine and containing 120 μCi of [^{35}S]methionine (Trans^{35}S-label; 1,179 Ci/mmol; ICN Biomedicals Inc.) was added. Monolayers were incubated in this medium for 4 h, and then cytoplasmic extracts were prepared in reticulocyte standard buffer–1% Nonidet P-40 (NP-40) and immunoprecipitated with anti-PVR antibody as described previously (16). Duplicate monolayers were treated for 8 h with tunicamycin (1 μg/ml; Boehringer Mannheim Biochemicals) at 40 h postinfection; labeling with [^{35}S]methionine was done as described above in the presence of 1 μg of tunicamycin per ml.

After immunoprecipitation, proteins were fractionated in 15% polyacrylamide-sodium dodecyl sulfate (SDS)-gels, stained with Coomassie blue, impregnated with Autofluor (National Diagnostics), dried, and exposed to Kodak X-Omat AR film with an intensifying screen for 1 to 3 days.

Solubilization of PVR and neutralization assays. Confluent monolayers of *S. frugiperda* cells in 15-cm dishes were infected with either AcNPV or AcPVR at an MOI of 10 for 48 h as described previously (33). Monolayers were washed and scraped into 1 ml of phosphate-buffered saline; cells were centrifuged for 10 s at 12,000 × *g*, suspended in (200 μl/dish) of reticulocyte standard buffer–1% NP-40, incubated for 10 min on ice, and centrifuged at 12,000 × *g* for 2 min to remove nuclei. Cytoplasmic extracts from two 15-cm dishes were diluted to 4 ml in phosphate-buffered saline and centrifuged at 100,000 × *g* for 1 h at 4 °C in a Beckman SW50.1 rotor. The supernatant was filtered through 0.2-μm-pore-size nitrocellulose, named S100WT and S100PVR, respectively, and stored at −70°C.

For neutralization assays, 100 μl of Dulbecco modified Eagle medium (DMEM) containing 2 × 10^6 PFU of poliovirus P1/Mahoney was mixed with 100 μl of S100WT or S100PVR and incubated for 3 h at 4°C and then for 30 min at either 4 or 37°C. Poliovirus titers were determined by plaque assay on HeLa cells (17). For plaque assay, adsorption was carried out for 15 min at 23°C and then for 1 h at 37°C or for 15 min on ice and then for 2 h at 4°C. Some samples were treated with 200 μl of 12 M LiCl for 1 h at 4°C before plaque assay.

Alteration assays. Poliovirus P1/Mahoney was propagated in confluent monolayers of HeLa cells in 15-cm plastic cell culture dishes (17). Cells were infected at an MOI of 10, labeled 4 h postinfection with 200 μCi of [^{35}S]methionine (Tran^{35}S-label; 1,179 Ci/mmol) in 15 ml of methionine-free DMEM, and harvested after overnight incubation at 37°C. Poliovirions were pelleted from the cell culture supernatant by centrifugation at 45,000 rpm in a type 60 rotor, suspended in NTE buffer (150 mM NaCl, 10 mM Tris hydrochloride [pH 7.4], 1 mM EDTA, pH 8.0), treated overnight with 1% SDS at room temperature, and purified by centrifugation in a linear gradient of 15 to 30% sucrose in NTE buffer. Gradients were centrifuged for 2 h at 40,000 rpm in an SW41 rotor at 4°C and fractionated. Fractions containing 160S particles were identified by scintillation counting, pooled, and stored at −70°C. Approximately 10^5 cpm of 160S particles was treated with 0.3 ml of S100PVR, incubated for 2 h at 4°C, and subsequently incubated for 1 h at either 0 or 37°C. Samples were then treated with 1 volume of either NTE buffer or 12 M LiCl for 3 h at 4°C before centrifugation in 15 to 30% sucrose gradients as described above. Untreated 160S particles and 80S particles obtained by heating 160S particles for 20 min at 56°C in 0.05% NP-40 (20) were centrifuged in a parallel gradient as markers.

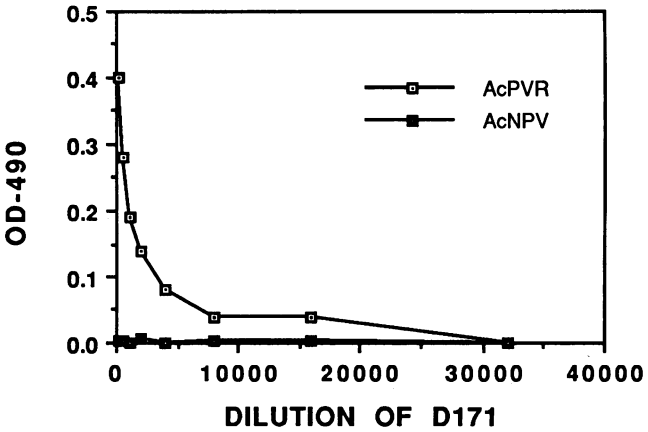

FIG. 1. Enzyme-linked immunosorbent assay for PVRs in AcPVR-infected *S. frugiperda* IPLB-SF-21 cells. Confluent monolayers of IPLB-SF-21 cells in 96-well plates were infected with either AcNPV or AcPVR at an MOI of 10. After 48 h at 28°C, duplicate wells were treated with twofold serial dilutions of D171 and stained with anti-mouse polyvalent immunoglobulins-peroxidase; and optical density at 490 nm (OD-490) was determined.

RESULTS

Expression of PVR in insect cells. To provide levels of PVR required for detailed studies on its structure and interaction with virus, we expressed the protein in insect cells by using a baculovirus vector. A recombinant baculovirus, AcPVR, was constructed that contained cDNA encoding the PVR. Southern and Northern (RNA) blot analysis of insect cells infected with AcPVR demonstrated that high levels of receptor cDNA and mRNA were produced during infection (data not shown). Expression of PVR at the insect cell surface was assessed by enzyme-linked immunosorbent assay, using antireceptor monoclonal antibody D171 (29). Insect cells infected with AcPVR but not AcNPV reacted with D171, indicating that the PVR was expressed at the cell surface (Fig. 1).

A poliovirus binding assay was used to determine whether the PVR expressed in *S. frugiperda* cells was functional. Poliovirus was adsorbed to insect cells infected with AcPVR or AcNPV, and the amount of infectious virus remaining in the cell culture supernatant was determined. The results clearly demonstrate that poliovirus bound to AcPVR-infected insect cells but not to AcNPV-infected insect cells

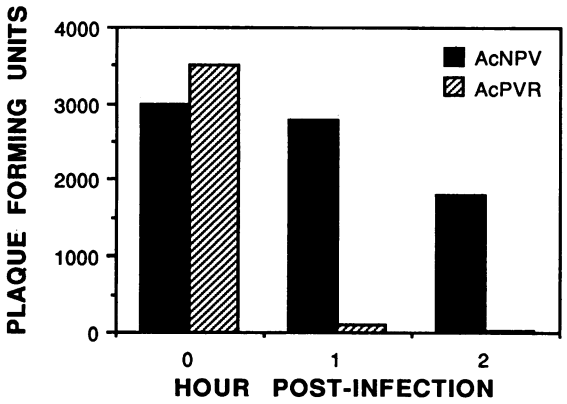

FIG. 2. Poliovirus binding to AcPVR-infected *S. frugiperda* IPLB-SF-21 cells. Confluent monolayers of IPLB-SF-21 cells in 10-cm plates were infected with either AcNPV or AcPVR at an MOI of 10. After 48 h at 28°C, infected monolayers were washed and inoculated with 10^4 PFU of poliovirus P1/Mahoney. Plates were incubated at 37°C for 2 h with swirling every 15 min, and samples were taken at 0, 1, and 2 h postinfection. Unbound virus was titrated on HeLa cell monolayers as described previously (17).

(Fig. 2). Poliovirus binding to AcPVR-infected insect cells was more efficient than binding to equal numbers of HeLa cells (data not shown), suggesting that the receptor was overexpressed in insect cells.

To determine the nature of the polypeptide expressed in AcPVR-infected cells, detergent extracts of *S. frugiperda* cells metabolically labeled with [^{35}S]methionine were immunoprecipitated with antiserum directed against a *trpE*-PVR fusion protein. The antiserum immunoprecipitated a diffuse polypeptide of 67 kDa, and a range of smaller products to 35 kDa, from AcPVR-infected but not from AcNPV-infected insect cells (Fig. 3, lanes 1 and 3). A polypeptide of 35 kDa was immunoprecipitated from AcPVR-infected cells that were treated with tunicamycin before labeling (Fig. 3, lane 4).

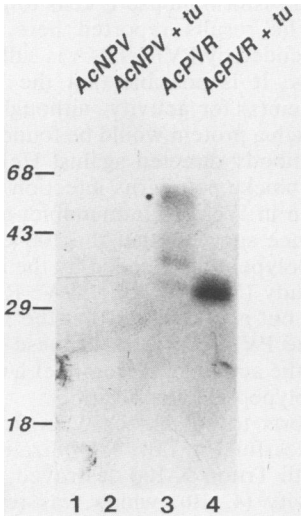

FIG. 3. Analysis of PVR expression in insect cells by immunoprecipitation. *S. frugiperda* cells were infected with AcNPV or AcPVR, and polypeptides were labeled with [^{35}S]methionine. Where indicated, cells were treated with tunicamycin (tu) before labeling. Polypeptides were immunoprecipitated from cytoplasmic extracts with anti-*trpE*-PVR fusion protein antiserum.

TABLE 1. Summary of neutralization experiments

Expt	Source of S100[a]	Incubation temp (°C)[b]	6 M LiCl[c]	Adsorption temp (°C)[d]	Virus titer (PFU/ml)[e]
1	WT	37	−	37	7.0×10^6
	PVR	37	−	37	9.0×10^3
2	WT	0	−	37	6.5×10^6
	WT	0	+	37	6.0×10^6
	PVR	0	−	37	1.1×10^6
	PVR	0	+	37	5.0×10^6
	PVR	37	−	37	4.5×10^3
	PVR	37	+	37	6.2×10^4
3	WT	0	−	4	1.5×10^6
	WT	37	−	4	1.2×10^6
	PVR	0	−	4	1.1×10^5
	PVR	37	−	4	1.9×10^3

[a] S100 was obtained from *S. frugiperda* IPLB-SF-21 cells infected with either AcNPV (WT) or AcPVR (PVR); 2×10^6 PFU of poliovirus P1/Mahoney in 100 μl of DMEM was incubated with 100 μl of S100WT or S100PVR in phosphate-buffered saline–0.1% NP-40.

[b] All samples were incubated for 3 h at 4°C and then for 30 min at the temperature indicated.

[c] After incubation, 200 μl of 12 M LiCl was (+) or was not (−) added.

[d] Adsorption was done for 15 min at 23°C and then for 1 h at 37°C or for 15 min on ice and then for 2 h at 4°C.

[e] Determined by plaque assay on HeLa cells (17).

Solubilization of PVR from insect cells. As a first step toward structural characterization, the PVR was solubilized from the membranes of insect cells by using NP-40. An S100 fraction from 10^8 *S. frugiperda* cells infected with AcNPV (S100WT) or AcPVR (S100PVR) was prepared. To determine whether solubilized PVR was biologically active, its ability to bind virus and neutralize infectivity was determined. Equal volumes of poliovirus (2×10^6 PFU in 100 μl of DMEM) and S100WT or S100PVR were mixed and incubated for 3 h at 4°C and then for 30 min at 37°C. Incubation with S100PVR reduced the viral titer nearly 1,000-fold compared with incubation with S100WT (Table 1, experiment 1).Increased reduction in viral titers was observed with more concentrated S100 (data not shown).

Mechanism of neutralization. It was of interest to determine the mechanism of poliovirus neutralization by solubilized PVR. Binding of poliovirus to cells at 4°C is reversible with 6 M LiCl or 0.4% SDS (12, 24). In contrast, when cell-bound virus is incubated at 37°C, irreversible loss of infectivity results. This phenomenon is called viral eclipse and is mediated by the conversion of virions to altered particles (12). The altered particles sediment at 135S (versus 160S for native virions), have lost VP4, and are H antigenic, protease sensitive, and noninfectious (7, 19). To determine whether neutralization mediated by the solubilized PVR was due to reversible binding or alteration, poliovirus was incubated with S100PVR at 4°C for 3 h; half of the sample was then incubated at 0°C, and half was incubated at 37°C. Before titration of virus infectivity, part of each sample was treated with 6 M LiCl. Incubation at 0°C with S100PVR reduced the viral titer sixfold compared with incubation with S100WT (Table 1, experiment 2). This neutralization was reversed by treatment with 6 M LiCl and was therefore due to blocking receptor attachment sites on the virion. Incubation with S100PVR at 37°C reduced infectivity 1,400-fold, and treatment with 6 M LiCl only partially restored infectivity (Table 1, experiment 2). Therefore, neutralization by solubilized PVR at 37°C appears to result from generation of altered particles. Detergent is not required for alteration, since neutralization activity of the solubilized receptor was maintained after sedimentation through a 5 to 20% sucrose

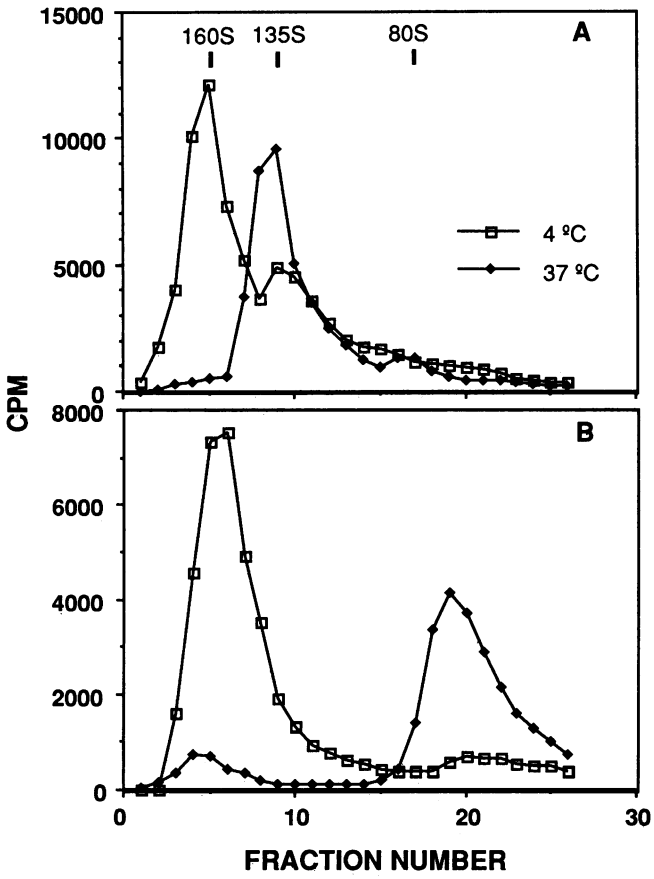

FIG. 4. Sedimentation analysis of poliovirions neutralized with solubilized cellular receptors. Approximately 10^5 cpm of 160S particles was treated with S100PVR, incubated for 2 h at 4°C, and subsequently incubated for 1 h at either 0 or 37°C. Samples were then treated with 1 volume of either NTE buffer (A) or 12 M LiCl (B) before centrifugation in 15 to 30% sucrose gradients. Untreated 160S particles and 80S particles obtained by heating 160S particles for 20 min at 56°C in 0.05% NP-40 (9) were centrifuged in a parallel gradient as markers.

gradient in the absence of NP-40 (data not shown). When virus and S100PVR were incubated at 4 or 37°C and adsorbed to HeLa cells at 4°C, infectivity was reduced 10- and 1,000-fold, respectively (Table 1, experiment 3), indicating that neutralization mediated by blocking virus attachment sites with solubilized receptors is less efficient than eclipse-mediated neutralization.

The sedimentation characteristics of particles resulting from incubation of poliovirus with S100PVR at 37°C were examined to determine whether 135S particles were formed. Virus was labeled with [^{35}S]methionine, purified by sucrose gradient centrifugation, and incubated with S100PVR at different temperatures and salt concentrations. The sedimentation patterns were analyzed at 4°C in 15 to 30% sucrose gradients (Fig. 4). Incubation of poliovirus and S100PVR at 37°C produced particles that sedimented at 135S. After incubation of poliovirus and S100PVR at 4°C, the bulk of poliovirions remained unaltered, migrating at 160S (Fig. 4A). The altered virus that appeared after incubation at 4°C might have resulted from either an inefficient eclipse of poliovirions at that temperature or manipulation of the samples during the experiment.

As described above, incubation of poliovirus with S100PVR at 37°C and subsequent treatment with 6 M LiCl for 3 h on ice did not fully restore infectivity. Sedimentation analysis of the product of this treatment indicated the formation of 80S and smaller particles (Fig. 4B). This finding is in agreement with the observation that eclipsed particles are labile in high salt (15). The 160S particles found in this sample probably represent unaltered virions, accounting for the residual infectivity observed (Table 1). As expected, there was no change in the sedimentation coefficient of poliovirions incubated at 4°C with S100PVR and then treated with 6 M LiCl, because unaltered poliovirions are resistant to high salt (Fig. 4B). The altered virions (135S) that result from binding at 4°C (Fig. 4A) were disrupted by-high salt treatment and sedimented slower than infectious particles (Fig. 4B). Thus, the interaction between poliovirions and solubilized or membrane-bound receptor is similar with respect to the temperature requirements for viral eclipse and the sedimentation coefficient and lability in high salt of the altered particles.

DISCUSSION

These experiments demonstrate that the PVR can be expressed on the surfaces of insect cells in a form that can bind poliovirus. Immunoprecipitation with anti-PVR antiserum revealed that the PVR is synthesized in insect cells as a heterogeneous glycoprotein with a maximum molecular size of 67 kDa. Treatment of AcPVR-infected insect cells with tunicamycin eliminated the polypeptide heterogeneity, and a single polypeptide of 35 kDa was immunoprecipitated. The 35-kDa species presumably represents the unglycosylated protein, whose predicted molecular size is 43 kDa (28). The difference between the observed and predicted sizes of the protein may reflect removal of the N-terminal signal sequence or anomalous electrophoretic mobility. These results indicate that the PVR is N glycosylated to about 50% of its molecular mass, but it is not known whether this modification is required for virus-binding activity.

The PVR cDNA used in this work encodes a polypeptide that is able to transform mouse L cells to poliovirus susceptibility (28). The results reported here suggest that the polypeptide encoded by PVR cDNA is sufficient to bind and alter poliovirus. It is possible that the PVR requires an additional protein(s) for activity, although we believe it is unlikely that such a protein would be found in insect cells. A monoclonal antibody directed against HeLa cells has been described that blocks poliovirus infection and reacts with a 100-kDa protein in Western immunoblot analysis (32). Preliminary evidence suggests that the 100-kDa protein is not related to the polypeptide encoded by the PVR cDNA clone used in this study (M. Shepley and V. Racaniello, unpublished results), but is is possible that the 100-kDa protein is a subunit of the PVR. Answers to these questions require purification of the active PVR from HeLa cells and determination of its polypeptide composition.

Previous efforts to solubilize PVRs from cultured cells were not successful (5, 13). Solubilization of HeLa cell membranes with Triton X-100 destroyed virus-binding and alteration activity (4, 10), which was restored only after removal of detergent by dialysis (4). However, these authors did not demonstrate that the virus-binding and alteration activity of the extracts remained soluble after dialysis. When a solid-phase assay was used to detect virus binding, solubilization of the PVR from membranes was achieved by using deoxycholate (18). However, the ability of this form of

the PVR to alter poliovirus particles was not reported. Solubilized cytoplasmic extracts prepared from HeLa cells in the same way as the extracts prepared from AcPVR-infected cells failed to neutralize poliovirus infectivity (data not shown). The reason for the difference between HeLa cells and AcPVR-infected insect cells is not known but may be related to the observation that PVR is expressed in insect cells at much higher levels than in HeLa cells. Results of binding assays with radiolabeled virus indicate that poliovirus binding to AcPVR-infected insect cell membranes is 100 to 1,000 times more efficient than binding to equal amounts of HeLa cell membranes (data not shown).

Several lines of evidence suggest that altered particles might be intermediates in poliovirus entry. Altered particles similar to those eluted from cells have been found within cells early after infection (7, 19). The antiviral compound arildone binds to virions and appears to inhibit infectivity by preventing alteration, suggesting that this step is important for infectivity (2, 27). Altered particles are more hydrophobic than native virions (20, 22, 23) as a result of a conformational alteration and exposure of the N terminus of VP1 (9). The hydrophobicity of altered particles might serve to embed the virus in the plasma membrane, providing a mechanism for passage of the viral genome into the cytoplasm.

The actual route of entry that leads to productive poliovirus infection has not been established. It has been suggested that the mechanism of poliovirus penetration and uncoating resembles that of enveloped viruses such as Semliki Forest virus and influenza virus (22, 23, 36). These viruses penetrate the cell by absorptive endocytosis. Acidification of the endosomes induces a conformational change in a spike glycoprotein that leads to fusion of the viral and endosome membranes, resulting in release of the viral genome into the cytoplasm. Shortly after infection, poliovirus particles can be seen in coated pits and endosomes, suggesting that entry occurs by endocytosis (35). It has been proposed that upon acidification of the endosome, the virion is altered, and the resulting hydrophobic particle then fuses with the endosome membrane, leading to release of the viral RNA into the cytoplasm (30). However, the results of experiments with inhibitors of endosome acidification are conflicting (22, 23, 36; M. Gromeier and K. Wetz, Abstr. 6th Meet. Eur. Study Group Mol. Biol. Picornaviruses, G21, 1989). Furthermore, the results described here demonstrate that virion alteration may occur at neutral pH in the absence of membranes. These findings suggest that viral eclipse may occur at the cell surface, after the virus particle has bound to a receptor. Perhaps cell-surface alteration results in anchoring of particles to the plasma membrane via exposed hydrophobic domains (9, 20–23; Gromeier and Wetz, Abstr. 6th Meet. Eur. Study Group Mol. Biol. Picornaviruses, 1989). In support of this notion, poliovirus has been observed free in the cytoplasm shortly after adsorption (6). Uncoating of the viral RNA may then occur at the plasma membrane or within the cytoplasm. Alternatively, endocytosis of the altered particle and additional modification may be required for uncoating of the viral genome. It is also possible that the small number of unaltered viruses which enter cells (35) could contribute to the initiation of the infection and therefore constitute an alternative pathway.

The observation that poliovirus alteration may be induced by protein-protein interaction at neutral pH will be useful for sorting out the mechanism of entry of this virus. The ability to produce large quantities of altered virions will enable biochemical and virological analysis of the particles and determination of their crystallographic structures. Comparison of the structures of altered and native poliovirus (11) will provide information on the basis for the structural transition.

Soluble CD4 (T4) protein can block human immunodeficiency virus type 1 infection by binding to gp120 and inhibiting virus attachment (1, 3, 8, 14, 34). Here we have shown that a solubilized cell receptor for poliovirus inhibits infection by two mechanisms: blocking the attachment site on the virion and inducing an irreversible alteration (eclipse) of the viral particle which destroys its infectivity. It may therefore be possible to use soluble cell receptors, or fragments of such receptors, as antipicornaviral agents. Recently it was demonstrated that a soluble form of intercellular adhesion molecule 1, the cellular receptor for rhinovirus, protects HeLa cells against rhinovirus infection (26). It will be of interest to determine whether this protection is mediated by blocking the viral attachment sites, by alteration of the viral particles, or by both mechanisms.

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ADDENDUM IN PROOF

M. Gromeier and K. Wetz (J. Virol. **64**:3590–3597, 1990) have provided evidence that poliovirus uncoating is independent of low pH.

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