The poliovirus receptor: a hook, or an unzipper?
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The cell receptor for poliovirus may be more than a simple ‘snare’ that attaches virus to cells. Recent results indicate that receptor binding may cause conformational changes in the virus that lead to uncoating of the viral RNA.

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All viruses must bind to a cell surface receptor to initiate infection. Some viruses, such as influenza virus, appear to use the cell receptor as a tether to hold the virus in place while the hemagglutinin mediates membrane fusion in response to low pH [1]. Many enveloped viruses undergo fusion with cell membranes at neutral pH, and the interaction with the cell receptor may trigger conformational changes in viral glycoproteins that convert them to fusogenic forms [2]. Non-enveloped viruses have the particularly difficult problem of how to transport the viral nucleic acid through a protein shell and across the cell membrane. Adenovirus is brought into the endocytic pathway by its cell receptor, but the receptor does not appear to participate in the stepwise disassembly of the particle that subsequently occurs [3]. It has been known for many years that the interaction of poliovirus with susceptible cells leads to the production of a conformationally altered particle [4] that is believed to be an intermediate in cell entry [5]. The determination of the three-dimensional structure of the viral capsid [6] and identification of the cell receptor for poliovirus [7] have lead to a better structural understanding of the virus–receptor interactions that lead to cell entry.

The cell receptor for poliovirus
The capsid of each of the three serotypes of poliovirus consists of sixty copies each of four viral proteins, VP1, VP2, VP3 and VP4. The poliovirus receptor (PVR), which is used by all three viral serotypes to initiate infection of cells, is a novel member of the immunoglobulin superfamily [7]. The PVR polypeptide contains an N-terminal signal sequence, three extracellular immunoglobulin (Ig)-like domains, a transmembrane domain, and a cytoplasmic tail. Alternative splicing results in two mRNAs encoding polypeptides of 392 and 417 amino acids: the two forms differ in the lengths of their cytoplasmic domains. Both forms of PVR function as receptors for poliovirus. The predicted molecular sizes of the two polypeptides are 43 and 45 kDa, respectively, although post-translational modification in HeLa cells produces a predominant species of 80 kDa [8].

Two human genes related to PVR, PRR1 and PRR2, have been identified, although it is not known if the encoded polypeptides function as poliovirus receptors [9,10]. A mouse homolog, MPH, does not bind poliovirus [11], including those that are adapted to grow in mice (Y Dong and VR, unpublished data). The cellular functions of PVR, PRR1, PRR2 and MPH are unknown although, like many members of the Ig superfamily, they may play a role in cell adhesion and recognition. The cytoplasmic domain of one isoform of PVR is phosphorylated at serine, possibly by calcium-calmodulin kinase II [12], and several protein kinases bind to and phosphorylate the cytoplasmic domain of MPH (Y Dong and VR, unpublished data). Identification of these protein kinases should provide clues about the normal role of PVR and MPH in the cell.

The role of PVR in uncoating of viral RNA
Shortly after poliovirus binds to cell surface PVR, it releases its RNA genome into the cytoplasm. PVR is likely to play a role in the uncoating step, as suggested by its ability to induce dramatic structural changes in the virus particle. When poliovirus is bound to cells at 37°C, a large proportion of the virus is eluted as a conformationally altered form known as the A particle [4]. These particles contain infectious RNA, but they differ from native virus in their sedimentation coefficient (135S compared with 160S for native virions), their increased sensitivity to detergent and proteinases, and the absence of VP4 [5]. The N terminus of VP1, normally on the interior of the virion, has been translocated to the surface, making the capsid hydrophobic. Conversion of poliovirus to 135S particles can also be accomplished in solution by incubation with detergent extracts of insect cells expressing PVR [13], or with soluble PVR released into the culture medium from expressing cells [14]. It is likely that PVR is sufficient for 135S particle formation, although this possibility has not yet been tested with the purified protein.

The A particle has been proposed to be an essential intermediate in the entry of poliovirus into cells [5]; see the article by James Hogle in this issue of Structure. The N terminus of VP1 may form an amphipathic helix that inserts into the cell membrane, producing a pore through which the viral RNA may leave the capsid. To determine the role of 135S particle formation in poliovirus replication, we took advantage of the observation that A particles are
not formed at temperatures below 33°C [15], and determined whether poliovirus could replicate at 25°C (A Dove and VR, unpublished data). Our findings indicate that the Mahoney strain of type 1 poliovirus, P1/Mahoney, is unable to grow at 25°C, but cold-adapted (CA) mutants are readily selected at this temperature. CA mutants replicate efficiently at 25°C without forming 135S particles (they do form 135S particles at 37°C). The CA phenotype does not map to the capsid-coding region of the viral genome, but rather to a central region encoding non-structural proteins. This suggests that the block to replication in WT P1/Mahoney occurs after the stage of cell entry, for example in RNA replication, proteolytic processing, or even assembly. In support of this hypothesis, when the entry steps are by-passed by transfection of viral RNA into cells, CA viral RNA replicates at 25°C, but WT RNA does not.

These results suggest that formation of 135S particles is not required for poliovirus replication. The altered particle might be a dead end and perhaps a less drastic PVR-induced conformational change might be the true intermediate in RNA uncoating. The ability to study a productive poliovirus infection at 25°C, in the absence of 135S particle formation, should enable the identification of such structural changes. We are left with the intriguing question of why poliovirus produces so many nonfunctional 135S particles; perhaps it is the price that must be paid for the ability to undergo receptor-mediated structural changes. Formation of 135S particles has been largely studied in cultured cells; in vivo, where the accessibility and/or level of cell receptors might be limited, it may be that fewer A particles are generated.

**PVR sequences that mediate virus binding**

To fully understand how the poliovirus–PVR interaction initiates cell entry, a detailed picture of how the virus and receptor combine is required. Ultimately, resolution of a virus–receptor complex will be needed, but the results of genetic analyses have already provided some insight into the interaction. The binding site for poliovirus appears to be contained within domain 1 (Fig. 1), which can bind poliovirus when expressed on the cell surface either alone or linked to other domains from CD4, ICAM-1 or MPH (reviewed in [16]). Virus does not bind as well to domain 1 as it does to native PVR, suggesting that domains 2 and 3 contribute to the interaction either directly or by influencing the structure of domain 1. Several laboratories have carried out mutational analyses on PVR domain 1 to identify the putative contact point, and the results show that three main sites are important for poliovirus binding (Fig. 1): the C–C′ loop through the C′′ strand; the border of the D strand and the DE loop; and the G strand. A mutation at the beginning of the F strand also reduces virus binding, probably by altering domain structure. Mutagenesis of other loops and strands has not revealed other regions that are important for binding.

These studies indicate that the C′–C′′ ridge is likely to be the main part of PVR that contacts poliovirus. The homologous part of CD4 plays a major role in the interaction with human immunodeficiency virus type 1 (reviewed in [17]). The DE loop of domain 1 may also contact poliovirus, but the G strand is more distant and not likely to be a component of the binding site. Consistent with this hypothesis is the observation that substitution of the region comprising PVR residues 70–100, which contains the C′–C′′ ridge (Fig. 1), into the corresponding region of MPH produces a chimeric receptor that can be recognized by type 1 but not types 2 and 3 poliovirus (Y Lin and VR, unpublished data). This result suggests that the poliovirus-binding site on PVR is contained within this 30 amino acid segment, although contribution of conserved MPH residues to poliovirus binding cannot be excluded. Apparently, the three serotypes of poliovirus contact PVR slightly differently, a conclusion also drawn from studies of the G-strand mutation (Fig. 1), which abrogates binding of types 1 and 2 but not type 3 poliovirus [18].

**Viral capsid sequences that mediate PVR interaction**

When the three-dimensional structures of rhinovirus and poliovirus were solved, a 1.2 nm deep, 1.5 nm wide channel...
was noted surrounding the prominent peak at the fivefold axis of symmetry of the particle [6,19]. This channel was called the canyon, and was proposed to be the receptor-binding site for rhinovirus 14 [19]. A model of the interaction of human rhinovirus 16 with its soluble receptor, ICAM-1, indicates that ICAM-1 does bind in the canyon [20]. Evidence that the canyon is the receptor-binding site in poliovirus comes from the study of two types of viral mutants: soluble receptor resistant (srr) mutants, and viruses adapted to utilize mutant PVRs. Detergent-solubilized PVR expressed in insect cells converts poliovirus to 135S particles, effectively neutralizing its infectivity [13]. Poliovirus mutants resistant to neutralization with soluble PVR have been selected; these mutants possess a range of binding defects to PVR [21,22]. Each srr mutant contains a single mutation, located either on the surface or the interior of the capsid. The surface mutations (white spheres in Fig. 2) are located in the canyon, and may be part of the contact site for PVR. Mutation at any one of eight surface residues decreases the binding affinity of poliovirus for PVR, indicating that multiple points in the virus–receptor interface contribute to binding. Mutations at internal capsid residues also reduce binding affinity. These residues are not likely to contact the receptor directly, but may affect the ability of the virus to bind to PVR with high affinity by altering the flexibility of the capsid. The proximity of several of the internal mutations to a hydrocarbon-binding pocket that appears to contain sphingosine [23] is consistent with this hypothesis. This pocket is believed to regulate the ability of the capsid to undergo receptor-mediated structural transitions [23].

Additional information on capsid sequences that control receptor interaction comes from the analysis of viral variants that are adapted to grow on cells expressing mutant forms of PVR that do not bind WT poliovirus P1/Mahoney [24]. PVR mutants d, g and i (Fig. 1) were constructed by substituting residues of PVR with corresponding residues from MPH. Because MPH is not a poliovirus receptor, this analysis identifies regions of PVR important for poliovirus binding [25]. Stable cell lines that express d, g or i mutants were derived from the mouse fibroblast line L. These cell lines cannot bind poliovirus, but viral variants were readily isolated that can utilize the mutant PVRs to infect cells. These adapted viruses can still utilize WT PVR to infect cells, and therefore possess an expanded receptor recognition. Sequence analyses and site-directed mutagenesis identified three different sites of mutations that are responsible for the adapted virus phenotype (Fig. 2; mutations shown as yellow spheres). Every adapted mutant contained a change at VP1 position 95, from proline to serine or threonine (P1095S/T; in this nomenclature, the first letter is the amino acid found in the WT virus, the next digit indicates the capsid protein VP1, VP2, VP3 or VP4, the next three digits the amino-acid position and the last letter the mutant amino acid). When either amino acid is introduced into WT virus by site-directed mutagenesis,
viruses are produced that can use all three mutant receptors. Position 95 of VP1 is located in the BC loop at the fivefold axis of symmetry, distant from the putative receptor contact site defined by srr mutations (Fig. 2). Although it is possible that this portion of the capsid also contacts PVR, the lack of allele specificity of the P109SS/T adapting mutation suggests that this residue is not likely to contact the mutated portions of PVR. This sequence might instead modulate the flexibility of the capsid and its ability to accommodate mutant receptors. Substitution of the entire VP1 BC loop with the sequence from the mouse-adapted Lansing strain of poliovirus type 2, P2/Lansing, enables P1/Mahoney to recognize an unidentified receptor in mice that cannot be used by the WT virus [26,27]. In this case, the VP1 BC loop of P2/Lansing may directly contact the mouse receptor, or it may impart to the capsid the flexibility to recognize a new receptor.

A second adapting mutation, V1160I, is located at the interface between protomers (the capsid subunit consisting of one copy each of VP1, VP2, VP3 and VP4), near the hydrophobic binding pocket of VP1. This mutation is not allele specific, and might also act by influencing the flexibility of the capsid. The V1160I mutation also allows P1/Mahoney to recognize a receptor in mice, thereby causing disease in that host. The mouse-adapted P2/Lansing contains isoleucine at amino acid 1160; curiously, it can utilize the g receptor but not the d and i receptors. A third adapting mutation, H2142Y, is located on the canyon wall near the receptor-binding site defined by the srr mutations. This mutation is allele specific, and will only correct the defect conferred by the d and g mutations, which are adjacent in PVR (Fig. 1). The nature of the amino acid at this location in the capsid may influence the contact point with PVR. The Leon strain of type 3 poliovirus contains tyrosine at 2142 but can only bind the receptor interact. Whether or not PVR, either in soluble form or associated with membranes, is sufficient to drive RNA uncoating can also be determined experimentally. Finally, the location in the cell at which the uncoating event occurs must be identified. These experiments will provide clues about how cell receptors participate in the uncoating of an icosahedral virus.

Does PVR work alone?

A mAb called AF3, which is directed against HeLa cells and blocks the binding of poliovirus to these cells in a serotype-specific manner [28], recognizes a specific isoform of the lymphocyte homing receptor CD44H [29]. This cell surface molecule is not a receptor for poliovirus, because expression of CD44H cDNA in PVR-negative mouse L cells does not confer the ability to bind poliovirus. Because the expression of the AF3 epitope is restricted to certain tissues that are susceptible to poliovirus infection, it was suggested that CD44 might be a determinant of poliovirus tissue tropism [28]. However, the results of growth curve analyses indicate that all three poliovirus serotypes multiply normally in cells that express PVR but not CD44, and the addition of CD44 by stable transformation has no effect on virus multiplication. Furthermore, the binding affinity constant for all three poliovirus serotypes is identical in the presence or absence of CD44. We conclude that CD44 is not required for poliovirus replication in cell culture. CD44H and PVR may be associated in the cell membrane, and AF3 may block poliovirus binding by its proximity to the virus-binding site on PVR.

Summary

These studies paint a hypothetical picture of the interaction of poliovirus with PVR, and how this union might initiate the uncoating steps. Contact between the virus and receptor occurs between capsid residues in the canyon and residues of the C–C′ ridge on domain 1 of PVR. High affinity binding is probably dependent both on the nature of the contact residues in the virus and the receptor, and on capsid residues at the protomer interface and in the interior that give flexibility to the capsid and allow it to conform to the receptor. Because the contact points in the canyon are located at the protomer interface, above the hydrocarbon-binding pocket, the interaction with PVR probably begins to destabilize the interface and weaken the affinity of sphingosine for the pocket. As the cell membrane wraps around the particle, additional PVR molecules bind to the capsid, perhaps releasing sphingosine and leading to complete destabilization of the capsid. The RNA might then emerge from an opening at the protomer interface. Crystallographic resolution of the virus–receptor complex will be required to demonstrate precisely how the virus and receptor interact. Whether or not PVR, either in soluble form or associated with membranes, is sufficient to drive RNA uncoating can also be determined experimentally. Finally, the location in the cell at which the uncoating event occurs must be identified. These experiments will provide clues about how cell receptors participate in the uncoating of an icosahedral virus.

References


