Interaction of the poliovirus receptor with poliovirus


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The structure of the extracellular, three-domain poliovirus receptor (CD155) complexed with poliovirus (serotype 1) has been determined to 22 Å resolution by means of cryo-electron microscopy and three-dimensional image-reconstruction techniques. Density corresponding to the receptor was isolated in a difference electron density map and fitted with known structures, homologous to those of the three individual CD155 Ig-like domains. The fit was confirmed by the location of carbohydrate moieties in the CD155 glycoprotein, the conserved properties of elbow angles in the structures of cell surface molecules with Ig-like folds, and the concordance with prior results of CD155 and poliovirus mutagenesis. CD155 binds in the poliovirus “canyon” and has a footprint similar to that of the intercellular adhesion molecule-1 receptor on human rhinoviruses. However, the orientation of the long, slender CD155 molecule relative to the poliovirus surface is quite different from the orientation of intercellular adhesion molecule-1 on rhinoviruses. In addition, the residues that provide specificity of recognition differ for the two receptors. The principal feature of receptor binding common to these two picornaviruses is the site in the canyon at which binding occurs. This site may be a trigger for initiation of the subsequent uncoating step required for viral infection.

Poliovirus (PV; family Picornaviridae, genus Enterovirus) is the etiologic agent of poliomyelitis, a human disease of the central nervous system. Effective vaccines against PV have been developed against the three known poliovirus serotypes: PV1, PV2, and PV3 (1). The structure of PV (2), like other picornaviruses such as rhinoviruses (3), consists of an icosahedral protein shell with an external diameter of ~300 Å that encapsidates a plus strand of RNA of roughly 7,500 bases. The capsid consists of 60 copies each of three larger proteins adopting an eight-stranded, antiparallel-a-barrel fold that is common to many other viral capsid structures (4).

Poliovirus infection is initiated by attachment to specific cell surface molecules. All three PV serotypes recognize the same cellular receptor molecule, the PV receptor (CD155) (5–7). In contrast, at least 78 of the more than 100 human rhinovirus (HRV) serotypes recognize intercellular adhesion molecule-1 (ICAM-1 or CD155-1) and CD54 as a cellular receptor, and other picornaviruses recognize a variety of different cell surface molecules (1). Although ICAM-1 is known to be involved in adhesion of lymphocytes to damaged or infected cells, the normal function of CD155 is uncertain.

Rhinovirus receptors originally were predicted to be long, narrow molecules that could bind to conserved residues within the canyon (3), the premise being that this might permit a strategy for the virus to escape host immune surveillance because bulkier neutralizing antibodies would be unable to enter the canyon. This prediction turned out to be correct with regard to the shape of the receptor for the major group of rhinoviruses (8, 9) and its site of attachment to the virus (10). However, the rationale of the prediction was questioned when the footprint of a neutralizing antibody was found to extend beyond the rims of the canyon (11), although naturally selected escape mutations that prevented antibody neutralization were located only on the viral surface outside the canyon (12). Genetic and mutational studies have mapped the CD155-binding site into the PV canyon (13–17), as also was done for the ICAM-1-binding site into the HRV canyon (cf. ref. 18).

CD155 and ICAM-1 are membrane-anchored single-span glycoproteins whose extracellular regions have three and five domains, respectively, with Ig-like folds (Fig. 1). Alternate splicing of the CD155 mRNA yields four different isotypes (α, β, γ, and δ), in which the extracellular domains are identical. CD155-β and -γ, however, are secreted, whereas the membrane-bound CD155-α and -δ serve as PV receptors (6, 7). The amino-terminal domain, D1, in both CD155 (13, 19, 20) and ICAM-1, contains the virus recognition site. Hence, virus attachment occurs at a site on the receptor distal from the plasma membrane, a property that may be important for successful initiation of infection of cells by virus.

Ig superfamily domains have a β-barrel fold in which all β-strands (labeled A to G) run parallel or antiparallel to the long axis of the domain. The fold of the CD155 D1 domain resembles (see “Model Fitting” in Materials and Methods) that of an Ig variable (V) domain (nomenclature reviewed by Chothia and Jones (21)) (Fig. 2), whereas the fold of the ICAM-1 D1 domain is intermediate (I) between the variable and constant (C) Ig folds. An Ig-like V domain has two extra β-strands, labeled C′ and C″, between β-strands C and D. Thus, the D1 domain of CD155 has 32 more residues than the D1 domain of ICAM-1. Furthermore, D1 in CD155 has two potential glycosylation sites, whereas the ICAM-1 D1 is unglycosylated. Absence of carbohydrate in CD155 D1 is known to enhance its binding to PV (16).
of CD155 interaction with PV and demonstrate that the mode of interaction is distinct from that observed in the HRV–ICAM-1 complexes. As predicted by mutagenesis studies (13–17), the footprint of CD155 on PV covers a region of the canyon similar to that found in HRV, but the CD155 molecule binds to PV in a different and more tangential orientation compared with the radial orientation of ICAM-1 bound to HRV.

Materials and Methods

Sample Preparation. All experiments were carried out with PV1(RIPO), a highly attenuated derivative of PV1(Mahoney) [PV1(M)] from which the cognate internal ribosomal entry site was exchanged with that of HRV2 (29). PV1(RIPO) has a wild-type type 1 capsid, expressing growth properties in HeLa cells at 37°C indistinguishable from that of PV1(M) (29).

A soluble derivative of CD155 was prepared by fusing the coding region of the 337 N-terminal codons of CD155 (including all three extracellular Ig-like domains) to the N-terminal coding region of human placental alkaline phosphatase (AP), using plasmid pAPtag2 (30) to yield plasmid pCD155-AP. The secreted fusion protein, CD155-AP (the calculated molecular mass in its unglycosylated form is 90 kDa), was expressed with this vector in 293 cells (human embryonic kidney cells transformed with adenovirus E1A,B genes) and was purified from cell supernatants (S.M., X.P., and E.W., unpublished results). Incubation of purified PV1(M) with purified CD155-AP reduced infectivity of the virus by about 5 orders of magnitude, whereas purified AP or mouse poliovirus receptor-related protein (mPRR2) had no effect on viral infectivity under the same conditions (S.M., X.P., and E.W., unpublished results).

Cryo-EM. Purified samples containing 40 μl of 8.75 mg/ml PV1 were mixed with 12 μl of 30 mg/ml CD155-AP at 4°C and incubated for 1 hr. Small (3.5-μl) aliquots of this mixture were adhered to carbon-coated electron microscope (EM) grids and vitrified in liquid ethane as described (31). Control samples of PV (i.e., not incubated with CD155-AP) were prepared similarly for cryo-EM. Electron micrographs were recorded on Kodak SO-163 film in a Philips CM200 FEG microscope (Philips, Eindhoven, the Netherlands), at a nominal magnification of 38,000 and a dose level of ~20 e–/Å². Micrographs were digitized on a Zeiss PHODIS microdensitometer at 14-μm intervals, which corresponds to 3.68 Å at the specimen.

An earlier reconstruction of the PV1 A particle (P. R. Chipman, R. R. Rueckert, T.S.B., and M.G.R., unpublished results) served as an initial model for determining the orientation of each projected particle by means of the model-based polar-Fourier transform method (32). Orientation refinement was monitored by means of correlation coefficients computed with real and reciprocal space data (33). The final correlation coefficients were a little lower than those generally observed in reconstructions of other, especially larger, icosahedral particles (cf. ref. 34) and in which particle overlap is not a problem, as was the case with the PV–CD155-AP complex sample. All maps were computed with Fourier–Bessel procedures, and eigenvalue spectra were used to monitor the conditioning of the linear, least-squares equations (35). The reso-
olution of each of the two maps (Table 1) was estimated by splitting the image data into two sets and comparing structure factors obtained in the separate reconstructions. The defocus level was calculated for each image and used to compute the phase-contrast transfer function corrections to include data to about 18-Å resolution in favorable cases (31). However, reasonable phase agreement was retained only to about 22-Å resolution between the two independent reconstructions.

Although the reconstructions were made with respect to a model of known hand, caution was exercised to ensure that the hand was not flipped during refinement. The hand was checked by noting the asymmetric features in the shape and environment of the canyon in the PV and HRV x-ray and EM structures (Fig. 3). A distinctive “landmark” is the “V” shape of the canyon within one icosahedral asymmetric unit (Fig. 3c), in which the base of the V lies clearly displaced to the “east” (see below for definition of north, south, east, and west). All reconstructions of both the HRV and PV samples displayed these same asymmetric features.

**Difference Map Calculations.** The x-ray crystal structure of PV1(M) [Protein Data Bank (PDB) ID code 2PLV] was used as a reference for scaling the pixel size and the density of the cryo-EM reconstructions. Structure factors were calculated from the x-ray coordinates with a “temperature” factor of 1,000 Å². These structure factors then were used to compute the reference electron density map to a resolution of 20 Å. The program MAMA (36) was used to zero out all electron density except for that within an annulus, defined by radii of 110–145 Å, to avoid the impact of the internal RNA and the external receptor in the canyon, neither of which is present in the x-ray structure. The program MAVE (36) was used to scale the PV–CD155-AP complex and PV EM maps to the x-ray map by using various radial shells to check the results for consistency (28). A difference electron density map then was computed by subtracting the scaled PV map from the scaled PV–CD155-AP map.

A difference map was calculated by subtracting the calculated contribution of the PV and CD155 atomic coordinates from the cryo-EM reconstruction map. This difference map identified the density corresponding to the glycosylation sites (Fig. 3d). Because of the increasing flexibility of the CD155 receptor at progressively larger distances from the virus surface, the scaling of the CD155 model to show the sites of glycosylation. The potential glycosylation sites are shown on the CD155 backbone (red).
of the canyon are present in both reconstructions. Nevertheless, it is probable that there are small differences in the virus structure, particularly when the differences in its aggregation tendencies are considered. The reconstruction also shows that the receptor is located, as anticipated, in the PV canyon at approximately the analogous site occupied by ICAM-1 when bound to HRV16 or HRV14 (10, 18, 28). However, the orientation of the CD155 molecule, relative to the surface of the virus, is completely different from that adopted by ICAM-1 on HRV (Fig. 3a and b). For an icosahedral asymmetric unit defined with a 5-fold axis in the “north” and with adjacent 3-, 2-, and 3-fold axes to the “south,” the ICAM-1 molecule leans toward the southwest, whereas CD155 leans toward the southeast. Furthermore, ICAM-1 adopts a more radial orientation with respect to the HRV surface, whereas CD155 is more tangential to the surface of PV, with much of the C,C,C’ surface of domain D1 contacting the viral surface (Fig. 5).

The density ascribed to CD155 decreased rapidly as a function of distance from the viral surface, and no significant density was detected in the difference map beyond a 260-Å radius. The mean densities for the PV protein capsid, the RNA, and the D1 domain of CD155 were about the same, indicating that roughly full occupation was obtained for CD155 at each of the 60 independent sites on the virus. The CD155 density by itself, as visualized in the difference map (Fig. 3d), showed densities for domains D1, D2, and D3 of 6, 4, and 2 SDs, respectively, with the largest noise peak being about 1 SD. The rapid decrease in density at progressively higher radii along the length of the receptor shows that there is considerable flexibility at the hinges between domains, which, therefore, allows the more distal domains greater freedom of motion and an ability to adopt different positions relative to the viral surface. Despite this property, domains D1, D2, and D3 were easily docked and fitted into the reconstructed difference density map (Fig. 3d). The elbow angles between pairs of domains were clearly apparent, with one of the potential N-glycosylation sites in D1 and two in domain D2, consistent with the features observed in the difference electron density (Fig. 3d). However, the large AP domain (53 kDa when unglycosylated) fused to the C terminus of the CD155 molecule was not seen in the cryo-EM reconstruction. From this observation, we presume that the fusion protein adopts highly variable orientations relative to the 60 binding sites on the virus surface, and this causes its density to average out upon enforcement of icosahedral symmetry during the three-dimensional reconstruction process. In contrast, the large AP moiety could be clearly seen as “lollipops” on unprocessed, negatively stained samples of the complex at a radius of about 280 Å from the viral center (data not

Fig. 4. Sequence alignment of human CD155 domain D1 with protein zero. Secondary structural elements are indicated, and potential glycosylation sites are marked with an asterisk. Residues implicated in binding to PV are marked with an X (15, 16) and a * (14, 17, 38) in the lines above the residue numbers. The color code for residues that are involved in binding to PV is the same as in Fig. 2.

Fig. 5. Stereoview of CD155 (yellow) docked onto PV, VP1, VP2, and VP3 coloring is blue, green, and red, respectively.

Model Fitting. In the absence of an atomic structure of CD155, it was necessary to find the closest available homologous models from the PDB for the fitting experiments. A BLAST (37) search (using the default values for gap penalties and other parameters) of the CD155 amino acid sequence (5) was conducted for each separate domain against all Ig superfamily entries in the PDB. The myelin membrane adhesion molecule—“human protein zero” (PDB ID code 1NEU)—gave the best fit for domain D1 (Fig. 4), which corresponded to an Ig-like V domain. An Ig light chain constant domain, derived from an antiidiotype Fab–Fab complex (PDB ID code 1CIC), was the best fit for domain D2 and corresponded to a constant Ig-like C2 domain. Finally, the insect immune protein (PDB ID code 1BIIH) was the best fit for domain D3 and corresponded to an Ig-like I domain. The sequence identity in each instance was about 25%. These homologous structures then were superposed to an Ig-like I domain. The sequence identity in each instance was about 25%. These homologous structures then were superposed to an Ig-like I domain.

Results and Discussion

The three-dimensional reconstruction of the PV–CD155-AP complex shows a good fit between the known PV structure and the cryo-EM density (Fig. 3d). Features such as the asymmetric shape of the canyon and the small protrusion on the south side of the model density to the EM reconstruction was performed separately for each of the three Ig-like domains. The difference map showed the sites of three of the carbohydrate moieties. The color code for residues that are involved in binding to PV is the same as in Fig. 2.

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shown). Nevertheless, the presence of the large AP at the C terminus demonstrated what had already been determined by constructing chimeric receptors (13, 19, 20)—that the domain closest to the virus surface must be domain D1 (12.6 kDa).

Domain D1 of human CD155 has potential N-glycosylation sites at residues Asn-105 and Asn-120. One of these sites is evident in the difference map (Fig. 3d). The site at Asn-120 lies fairly close to the rim of the canyon, whereas the site at Asn-105 is well outside of the canyon. However, if a different orientation of D1 had been assumed in the fitting procedure, it would have brought Asn-105 into the canyon, causing steric hindrance with the virus surface, further confirming the correct docking of the CD155 homology model into the cryo-EM density. Similarly, the two potential glycosylation sites in monkey CD155 (22) are positioned in the model so as not to cause any steric hindrance with the viral surface. In contrast, mPRR2 (7) has a potential glycosylation site corresponding to Gln-130, within the interface between CD155 and PV, suggesting that glycosylation at this site would contribute to the inability of mPRR2 to bind PV (7).

It may not be mere coincidence that the closest available homologous structure to domain D1 of CD155 is the peripheral nerve myelin protein zero. The presence of CD155 on neurons and of protein zero on the surrounding myelin sheath may indicate a recent evolution from a common precursor. The biological function of CD155 is not clear; however, available evidence suggests that CD155 may be in contact with residues from two neutralization antigenic sites on either side of the canyon (15, 16). Because the D1 domain of CD155 leans toward the virus surface, rather than being oriented radially like ICAM-1, much of its C,C,C face makes additional, extensive contact with the viral surface. In this respect, utilization of the C,C,C face by CD155 for interaction with its viral ligand is similar to CD4 with HIV (40, 41). With a 1.4-Å-radius probe as a basis for measurement, the CD155 footprint covers 1,300 Å² of the PV surface. In comparison, ICAM-1 only has a 900-Å² footprint on HRVs (28). The larger CD155 footprint can be attributed mostly to the contacts made between the C,C,C surface and the virus.

The north rim of the canyon is formed by the VP1 BC loop (residues 1,091 to 1,107) and the VP1 βE to αB loop (residues 1,166–1,169). These residues form a hydrophobic surface that interacts with an equally extensive hydrophobic surface (Fig. 6) on CD155 (residues 29–31 at the amino terminus of the βA strand, residues 54 and 55 at the beginning of the BC loop, and residue 135 near the beginning of the βG strand). Both PV loops that form the north rim of the canyon had been implicated correctly by mutagenesis studies as being involved in binding of CD155 (15).

The south rim of the canyon forms a hydrophilic surface by virtue of residues 2,138–2,142 and 2,172 in the “puff” (a large insertion in HRV receptor, in addition to differences in the specific site interactions required for binding. Binding of ICAM-1 to the site of the PV receptor has similar steric problems generated by the different elbow angle.

The footprint of CD155 on the surface of PV was established from the atomic model of the PV–CD155 complex by identifying those PV residues with atoms that lie within 4.0 Å from any CD155 atom in the modeled complex (Fig. 6). The viral and receptor surfaces involved in the interface are both in excellent agreement with mutational data (see below) and, hence, validate the accuracy of the model building. Unlike ICAM-1, which contacts primarily the floor and south wall of the HRV canyon, CD155 overlaps the north and south walls as well as floor of the canyon. Indeed, it has been suggested that CD155 may be in contact with residues from neutralization antigenic sites on either side of the canyon (15, 16). Because the D1 domain of CD155 leans toward the virus surface, rather than being oriented radially like ICAM-1, much of its C,C,C face makes additional, extensive contact with the viral surface. In this respect, utilization of the C,C,C face by CD155 for interaction with its viral ligand is similar to CD4 with HIV (40, 41).

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VP2 between strand βE and helix eB) and residues 1,223–1,226 in the GH loop of VP1. These residues contact the C strand of CD155, consistent with the mutational analysis of PV (17) and of CD155 (16, 38).

The floor of the canyon is formed primarily by residues in the GH loop of VP1 (1,228–1,235), the GH loop of VP3 (3,180), and the end of the VP1 BC loop (residue 1,109). These interact with residues (126–135) in the FG loop of CD155, consistent with the mutational analysis of the virus (14, 17) and of CD155 (15).

The viral surface in contact with the C, C′, C′′ surface of CD155 domain D1 is formed by the “knob” (residues 3,058–3,062, an insertion in β-strand B of VP3) and the C-terminal region of VP1 (residues 1,294–1,301). This assignment agrees with studies of CD155 mutants (15), although the involvement of the corresponding viral surface was not implicated in these studies. Contacts in this region are stabilized by complementary electrostatic interactions between Glu-71 in CD155 and Lys-3062 and between Lys-90 and Arg-91 in CD155 and Asp-1298. Analogous complementary interactions were found to be important in determining the specificity of ICAM-1 for the major receptor group of rhinoviruses (18, 28). Thus, the occurrence of some charge complementarity between PV and CD155 also may play a significant role in the recognition process.

Substantial evidence (28) suggests that the structures observed for the HRV–ICAM-1 complexes represent an initial recognition event. Only subsequently is the receptor likely to bind deeper into the canyon and thereby possibly compete out the lipid moiety in the VP1 pocket (23), which then leads to virus destabilization and progressive disassembly and release of the genomic RNA. CD155 binding may follow a similar pathway, as evidenced in the EM analysis of the virus (14, 17) and of CD155 (15). The floor of the canyon is formed primarily by residues in the GH loop of VP1. These residues contact the C strand of CD155, consistent with the mutational analysis of PV (17) and of CD155 (16, 38).

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