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MINIREVIEW

Cell Recognition and Entry by Rhino- and Enteroviruses

Michael G. Rossmann,*[,1] Jordi Bella,*[,2] Prasanna R. Kolatkar,*[,3] Yongning He,*
Eckard Wimmer,† Richard J. Kuhn,* and Timothy S. Baker*

**Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907-1392; and †Department of Molecular Genetics and Microbiology, School of Medicine, State University of New York at Stony Brook, Stony Brook, New York 11794-5222*

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I. VIRAL RECEPTORS

Unlike plant viruses, most mammalian, insect, and bacterial viruses attach to specific cellular receptors that, in part, determine host range and tissue tropism. Viruses have evolved to utilize a wide variety of cell-surface molecules as their receptors, which include proteins, carbohydrates, and glycolipids. Some viruses recognize very specific molecules, such as the intercellular adhesion molecule-1 (ICAM-1; CD54), the receptor recognized by a large group of rhinoviruses, whereas other viruses recognize widely distributed chemical groups, such as sialic acid moieties by influenza viruses. The tissue distribution of the receptor, in part, determines virus tropism and, hence, the symptoms of the infection. Similarly, species differences between receptor molecules can limit host range. For instance, only humans and apes are susceptible to rhinovirus infections, a property correlated with the inability of human rhinoviruses (HRVs) to bind to the receptor ICAM-1 molecule in other species.

Despite extensive similarities in sequence, structure, and physical properties among picornaviruses, suggesting evolution from a common ancestor (Rossmann *et al.,* 1985; Palmenberg, 1989; Rueckert, 1990), they nevertheless recognize a variety of receptors. Possibly a primordial virus was able to bind weakly to a large number of different molecules. Subsequently, different viruses evolved that were progressively more efficient at recognizing particular molecules as a means to infect specific cells. Indeed, the grouping of picornaviruses might suggest such a scenario. All polioviruses (PVs) appear to recognize human CD155 ["poliovirus receptor" (Mendelsohn *et al.,* 1989)] and many coxsackie B viruses recognize coxsackie-adenovirus receptor (CAR), whereas certain echoviruses recognize decay-accelerating factor (DAF; CD55). It is surprising, therefore, that rhinovirus serotypes can be divided into roughly three groups, each group recognizing a different cellular receptor molecule (Abraham and Colonno, 1984; Uncapher *et al.,* 1991). The receptor for the major group of rhinoviruses, ICAM-1, belongs to the immunoglobulin (Ig) superfamily (Greve *et al.,* 1989; Staunton *et al.,* 1989), whereas the receptor for the minor group is the low-density lipoprotein (LDL) receptor (Hofer *et al.,* 1994).

Receptor binding is just the first step in infection. The virus or maybe only the genome then enters the cell in a process that requires translocation of the viral genome or a subviral particle across the membrane into the cytoplasm, and sometimes into the nucleus. Since genome delivery requires or accompanies major rearrangements of the capsid structure, entry must be a tightly regulated process, which is triggered by the cell. The mechanism of entry, for example for enveloped viruses, requires fusion of the viral envelope with the limiting cellular membrane. Nonenveloped viruses such as picornaviruses (Rueckert, 1990) enter the cytoplasm in a manner that has not been well elucidated, though it must differ significantly in detail from the membrane-fusion strategy adopted by enveloped viruses.

II. PICORNAVIRUSES AND THE CANYON HYPOTHESIS

Picornaviruses are small, icosahedral, nonenveloped viruses with a plus-sense RNA genome. They are among the most common animal virus pathogens and include HRVs and PVs. High-resolution structures have been determined for a variety of HRV and PV serotypes by means of X-ray crystallography (Hogle *et al.,* 1985; Ross-

[1] To whom correspondence should be addressed.
[2] Present address: School of Biological Sciences, University of Manchester, 2.205 Stopford Building, Oxford Road, Manchester M13 9PT, United Kingdom.
[3] Present address: BioInformatics Centre, National University Hospital, Ground Floor, Lily Lobby, National University of Singapore, Singapore 119074.

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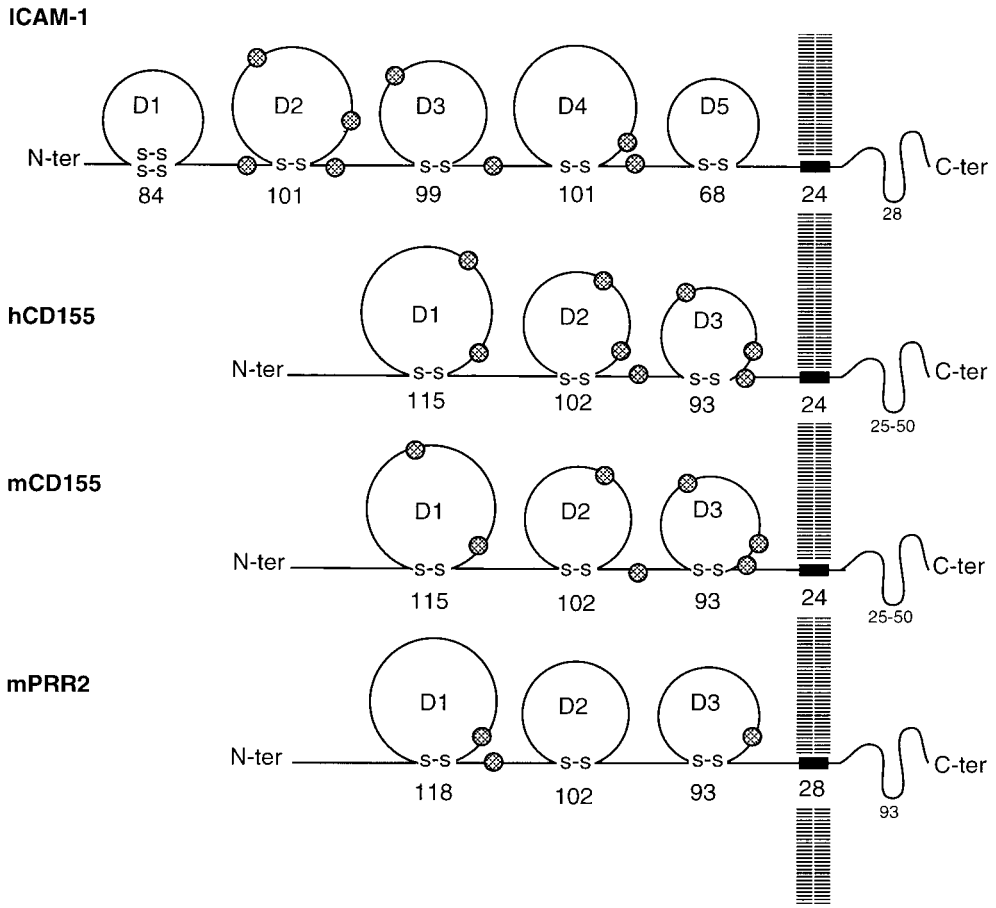

FIG. 1. Comparison of the mature structures of ICAM-1, the receptor for the major group of rhinoviruses, with the human PV receptor (hCD155), the monkey PV receptor (mCD155), and the murine poliovirus receptor-related protein 2 (mPRR2). Sites of glycosylation are indicated by shaded circles. The number of amino acids is shown for each domain. [Reprinted with permission from He *et al.* (1999) Copyright National Academy of Sciences.]

mann *et al.,* 1985). The capsids are structurally quite similar, but HRVs and PVs differ in both their pathology and stability (HRVs are unstable below pH 6). Thus, PVs can survive in the stomach and intestines, whereas rhinoviruses thrive primarily in the upper respiratory system. Virions are about 8.5×10^6 daltons in mass, have an external diameter of ~300 Å, and contain 60 protomers, each of which is made up of four polypeptides, VP1–VP4. The first three of these viral proteins reside on the exterior of the virus and make up its spherical protein shell. The three PV serotypes all recognize the same cellular receptor molecule, CD155 (Mendelsohn *et al.,* 1989; Koike *et al.,* 1990; Wimmer *et al.,* 1994). At least 78 of the more than 100 HRV serotypes recognize ICAM-1 as a cellular receptor, and other picornaviruses recognize a variety of different cell-surface molecules (Rueckert, 1996). Although ICAM-1 is known to be involved in adhesion of lymphocytes to damaged or infected cells, the normal function of CD155 is uncertain.

The capsids of rhino- and enteroviruses have a narrow surface depression ("canyon") that surrounds each of the 12 fivefold vertices. Rhinovirus receptors were predicted to be long, narrow molecules that could bind to conserved residues within the canyon (Rossmann *et al.,* 1985). This mode of binding would permit the virus to escape host immune surveillance because bulkier neutralizing antibodies should be unable to enter the canyon. This prediction turned out to be correct with regard to the site of receptor binding in the canyon and the shape of the receptor for the major group of rhinoviruses (Olson *et al.,* 1993; Kolatkar *et al.,* 1999) and for polioviruses (Belnap *et al.,* 2000a; He *et al.,* 2000). 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 (Smith *et al.,* 1996) even though naturally selected, escape mutations that prevented antibody neutralization were located on the viral surface well outside the canyon (Rossmann *et al.,* 1985; Sherry and Rueckert, 1985).

CD155 and ICAM-1 are membrane-anchored, single-span glycoproteins whose extracellular regions consist of three and five domains, respectively, each with Ig-like folds (Fig. 1). The amino-terminal domain, D1, in both CD155 (Freistadt and Racaniello, 1991; Koike *et al.,* 1991; Selinka *et al.,* 1991) and ICAM-1 (Staunton *et al.,* 1990; McClelland *et al.,* 1991; Register *et al.,* 1991) contains the

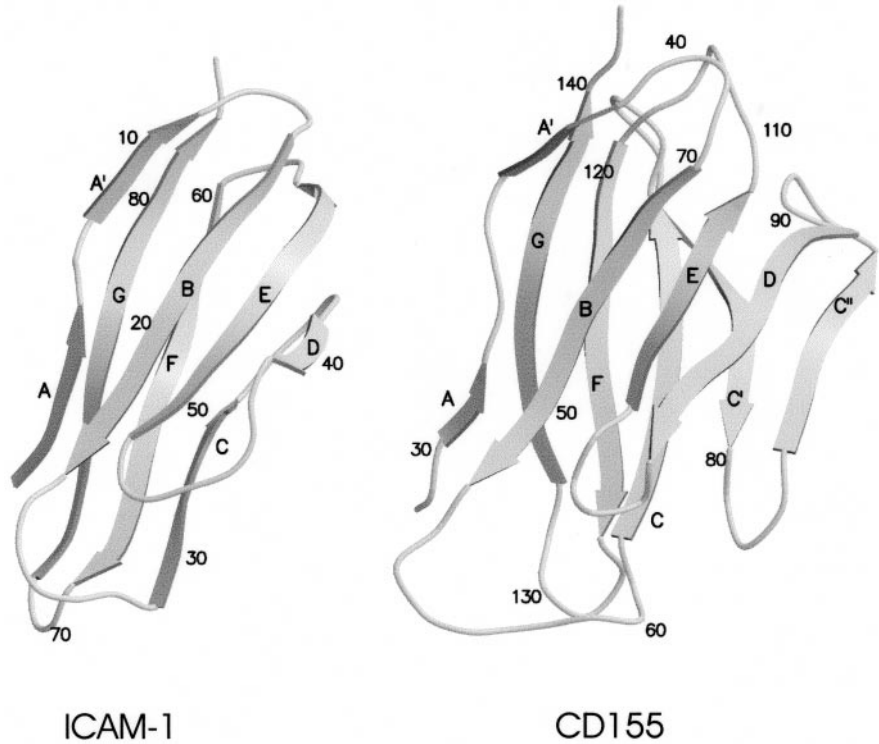

FIG. 2. (Right) The C_α backbone of domain D1 of CD155 based upon its homology to protein zero. (Left) The C_α backbone of domain D1 of ICAM-1 based upon its crystallographic structure determination. Labeling of the β-strands, the sites of potential glycosylation, and strategically numbered residues are indicated.

virus recognition site. Hence, virus attachment occurs at a site on the receptor that is distal from the plasma membrane. This property may be important for successful initiation of infection of cells by viruses and may reflect the enhanced ability of the N-terminal Ig domain to penetrate into the picornaviral canyon.

Ig superfamily domains have a structure that consists of a β-barrel fold in which all β-strands (labeled A–G) run parallel or antiparallel to the long axis of the domain. The fold of the CD155 D1 domain resembles that of an Ig variable (V) domain [nomenclature reviewed by Chothia and Jones (1997)], whereas the fold of the ICAM-1 D1 domain is intermediate (I) between the variable and constant (C) Ig folds (Fig. 2). An Ig-like V domain has two extra β-strands, labeled C′ and C″, between β-strands C and D. Thus, compared to ICAM-1, the D1 domain of CD155 has 32 more residues. D1 in CD155 also has two potential glycosylation sites, whereas the ICAM-1 D1

TABLE 1

Different ICAM-1 Fragments

Type	Residues	Domains	Mutations	Expression system	Glycosylation[a]	Crystal structure	Protein Data Bank id code
1	1–185	D1–D2	Wild type	CHO cells	Full, complex	(Kolatkar *et al.,* 1999)	1D3L
2	1–185	D1–D2	N103 → Q N118 → Q N156 → Q	Baculovirus-infected SF9 cells	Reduced, complex	(Bella *et al.,* 1998)	1IAM
3[b]	1–190	D1–D2	Wild type	Modified CHO cells Lec3.2.8.1[c]	Full, high-mannose	(Casasnovas *et al.,* 1998)	1IC1
4	1–453	D1–D5	Wild type	CHO cells	Full, complex	—	—

[a] The Asn to Gln mutations in type 2 ICAM-1 remove three out of four glycosylation sites in ICAM-1 D2; the mutant CHO cell line used for type 3 ICAM-1 expression produces mannose-only glycans with reduced heterogeneity.

[b] Two independent molecules per crystallographic asymmetric unit, referred to as A and B.

[c] (Casasnovas *et al.,* 1998).

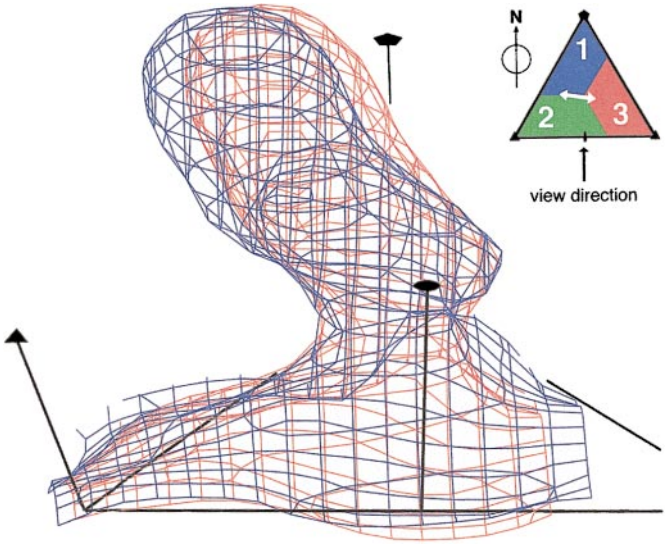

FIG. 3. Cryo-EM reconstructions for HRV16–type 1 ICAM-1 (in red) and HRV14–type 1 ICAM-1 (in blue) depict the density corresponding to the ICAM-1 fragments. Positions of the icosahedral symmetry elements are shown. A small angular difference in orientation of receptor relative to the viral surface indicates a slightly different binding of ICAM-1 to each serotype. The main direction of variation is depicted schematically with a white arrow in the asymmetric unit representation (inset), roughly parallel to the canyon depression. The edge of the canyon nearest to the fivefold axis is defined as the "north wall." The positions of VP1 (blue), VP2 (green), and VP3 (red) are also shown diagrammatically. [Reprinted with permission from Kolatkar *et al.* (1999) Copyright European Molecular Biology Organization.]

lacks glycosylation. Absence of carbohydrate in CD155 D1 is known to enhance its binding to PV (Bernhardt *et al.,* 1994).

Cell entry and uncoating are initiated when PV and HRV recognize their respective receptors (Koike *et al.,* 1992; Rossmann, 1994; Belnap *et al.,* 2000b). Purified, soluble receptor molecules, as well as the membrane-anchored receptor, convert infectious virions to altered ("A") particles (135S) (Hoover-Litty and Greve, 1993; Wimmer *et al.,* 1994). VP4 is absent in A particles, and the N-terminus of VP1 is externalized (Fricks and Hogle, 1990). Slightly longer incubation leads to the formation of 80S particles, which are devoid of the genomic RNA. It is uncertain, however, whether the 135S and 80S particles are intermediates in the uncoating pathway (Curry *et al.,* 1996; Dove and Racaniello, 1997; Arita *et al.,* 1998).

It has been suggested (Rossmann, 1994) that binding of the receptor might expel a lipid moiety that resides in a hydrophobic pocket within VP1, which is immediately underneath the floor of the canyon, thereby destabilizing the virion and, hence, initiating uncoating. Considerable experimental data now exist that support this mechanism: (i) antiviral compounds bound to the hydrophobic pocket in VP1 inhibit uncoating in HRVs and PVs (Fox *et al.,* 1986); (ii) these antiviral compounds preclude cell binding in many of the major-group HRVs (Pevear *et al.,* 1989, 1992); (iii) HRV antiviral escape mutants may in-

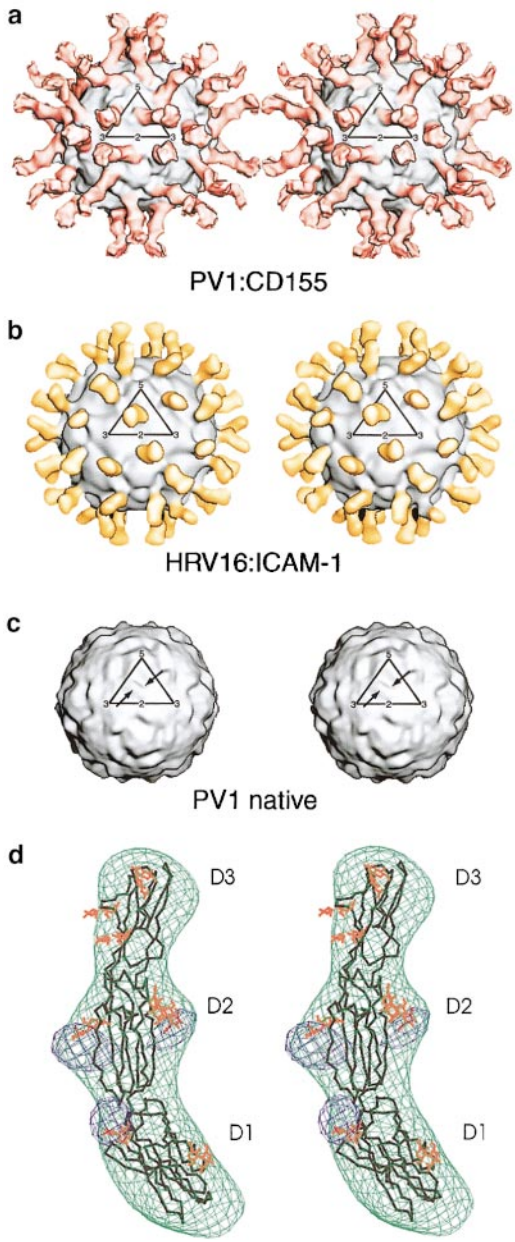

FIG. 4. (a) Stereo view of the cryo-EM reconstruction showing the complex of PV1(M) with human CD155. The outline of one icosahedral asymmetric unit is shown. Note that the receptor leans in a southeast direction. (b) Stereo view of the cryo-EM reconstruction showing the complex of HRV16 with its ICAM-1 receptor [from Kolatkar *et al.* (1999)]. The outline of one icosahedral asymmetric unit is shown. Here the receptor leans southwest. (c) Stereo view of a cryo-EM reconstruction of PV, also showing the icosahedral asymmetric unit. The asymmetric shape of the canyon is noted by the most southerly point situated slightly east of center (arrow) and the smaller peak southwest of the canyon (arrow). These features establish the correct hand of the reconstructions in (a) and (b) and are consistent with the X-ray results, where the absolute hand is known. (d) Density (green) representing one CD155 molecule fitted with the C_α backbone structure of the closest homologous structures found in the PDB for each of the three domains. The difference map (blue) between the cryo-EM density and the unglycosylated CD155 model shows the sites of glycosylation. Potential glycosylation sites are depicted on the CD155 backbone (red). [Reprinted with permission from He *et al.* (2000) Copyright National Academy of Sciences.]

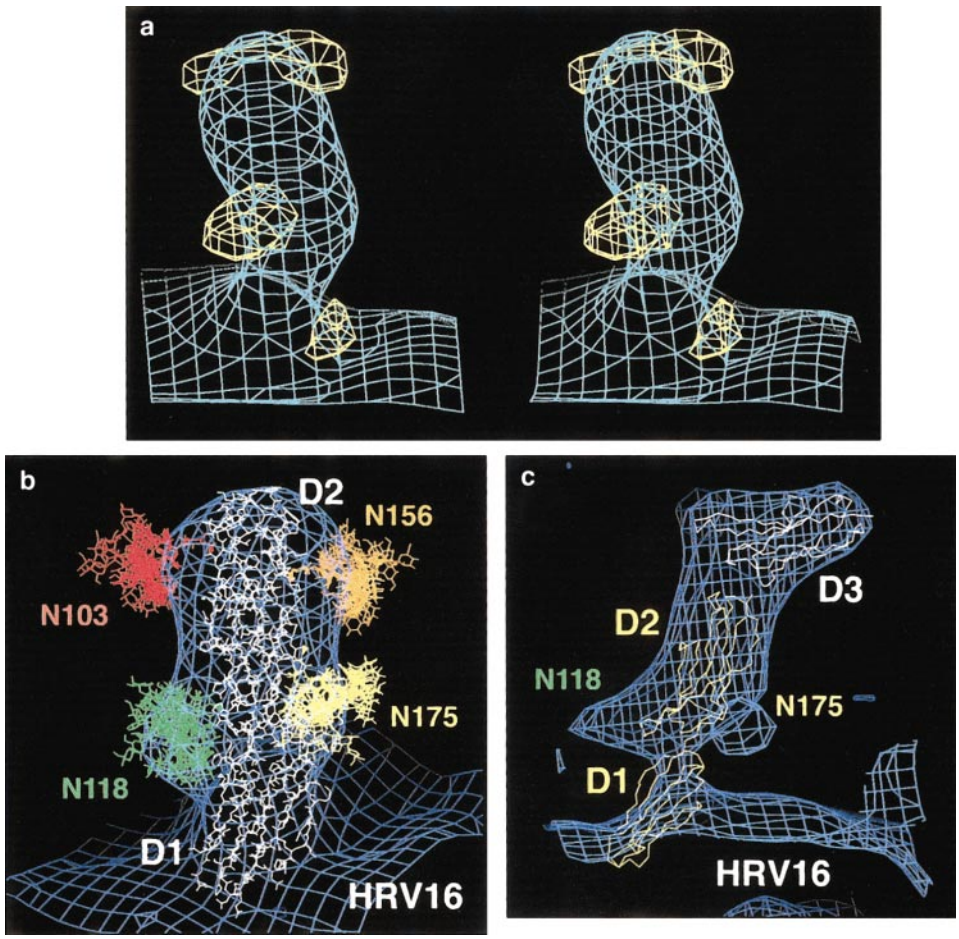

FIG. 5. (a) Stereo diagram of a portion of the HRV16–type 2 ICAM-1 cryo-EM reconstruction corresponding to the density (light blue) for the two-domain ICAM-1 fragment. Superimposed, in yellow, is the difference density map between HRV16–type 1 (fully glycosylated) and HRV16–type 2 (mostly deglycosylated) reconstructions. The density in the HRV16–type 2 reconstruction has been suitably scaled to account for the lower occupancy of the ICAM-1 fragments. (b) Fitting of the refined type 1 ICAM-1 model into the cryo-EM reconstruction of the HRV16–type 1 ICAM-1 complex. The protein is represented in grayscale, and the disordered carbohydrates are represented by an ensemble of conformations (yellow). (c) A C_α model of D1 to D3 of ICAM-1, manually fitted into the HRV16–type 4 ICAM-1 reconstruction. D2 coordinates were used to model domain D3 of ICAM-1. Additional lumps of electron density correspond to the predicted positions for two carbohydrate moieties on ICAM-1 D2, consistent with the difference density map shown in (a) and the refined ICAM-1 D1D2 model in (b). [Reprinted with permission from Kolatkar *et al.* (1999) Copyright European Molecular Biology Organization.]

volve changes in residues either at the surface of the canyon or in the hydrophobic pocket, thereby either increasing the affinity of the virus for its receptor or decreasing the affinity of antiviral compounds for the virus (Hadfield *et al.,* 1995); (iv) HRV14 is able to externalize both VP4 and the N-terminus of VP1 in a spontaneous, probably reversible manner ("breathing"), as shown by limited proteolysis followed by mass spectroscopy (Lewis *et al.,* 1998); (v) antibodies against internal epitopes on VP1 and VP4 cause PV neutralization, also suggesting a breathing mechanism for PVs (Li *et al.,* 1994); (vi) HRV14 breathing is largely inhibited by antiviral compounds (Lewis *et al.,* 1998); (vii) there is kinetic evidence for two binding modes of ICAM-1 on the surface of HRVs (Casasnovas and Springer, 1995); and (viii) complexes between HRVs and soluble receptors can be obtained as metastable entities that can be visualized by cryoelectron microscopy (cryo-EM), and subsequent changes in temperature, pH, or receptor concentration trigger an irreversible uncoating step (Hoover-Litty and Greve, 1993; Olson *et al.,* 1993). In spite of this seeming wealth of data, the precise details of the interaction of HRVs with ICAM-1 and PVs with CD155 and the sequence of events that lead to uncoating remain to be confirmed.

III. CRYO-EM OF VIRUS–RECEPTOR COMPLEXES

Cryo-EM and X-ray crystallography, in conjunction with atomic modeling, have been used to examine the interactions of ICAM-1 with two different HRV serotypes (Bella *et al.,* 1998; Kolatkar *et al.,* 1999) and the interactions of CD155 with PV serotype 1 (Belnap *et al.,* 2000a; Xing *et al.,* 2000; He *et al.,* 2000). The structure of each

complex was modeled at atomic resolution by fitting appropriate crystallographic structures of the virus and of the receptor to cryo-EM density maps. The fitting of each virus was a straightforward procedure because all that was required was, first, to orient the X-ray and EM structures to superimpose the known icosahedral symmetry elements and, second, to radially scale the EM map to match the X-ray structure. The EM and crystallographic density maps, when calculated to similar resolution limits, exhibit excellent agreement and, hence, validate the fitting process. Radial scaling of the cryo-EM density map to the crystallographic structure of the virus (Rossmann, 2000) compensates for uncertainties in the absolute magnification of EM images, which can vary up to several percent from the nominal values recorded at the time of microscopy (Olson and Baker, 1989). However, the fitting of crystallographically determined viral receptor structures to the corresponding density features in cryo-EM maps of virus–receptor complexes was a more difficult process.

The domain structures of ICAM-1 and homologous structures of CD155 are well known, but the elbow angles between the domains in each receptor can vary considerably. In addition, the atomic fitting procedure had to proceed such that steric clashes with the virus were minimized, though recognizing that formation of the complex might have been accompanied by conformational changes in either virus or receptor. The orientation and positional indicators, such as glycosylation sites, helped guide the accurate positioning of crystallographic models within the cryo-EM density maps. The combination of cryo-EM and crystallographic data yielded an accuracy of better than 2 Å in positioning individual receptor domains with respect to the virus surface. As the separation between consecutive C_α atoms in a polypeptide is 3.8 Å, the accuracy of positioning the receptor molecule was sufficient to identify the putative chemical interactions between the amino acids of the receptor and viral surface.

Interactions of various ICAM-1 derivatives (Table 1) with two rather different HRV serotypes are similar in that the orientations of the long ICAM-1 molecule relative to the viral surface differ by only 2 or 3°(Fig. 3). Interaction of CD155 with PV1 is very different from ICAM-1 with HRV (Belnap *et al.,* 2000a; Xing *et al.,* 2000; He *et al.,* 2000), although the two receptors bind to similar sites within the canyons (Fig. 4). ICAM-1 also binds into the coxsackievirus A21 canyon, but in a quite different orientation from that seen in HRV or of CD155 into the PV canyon (Xiao, Bowman, Baker, Kuhn, and Rossmann, unpublished results). Thus, the sites of receptor binding within the canyon are conserved among entero- and rhinoviruses, and the receptors are long, slender, flexible molecules, although their orientations on the viral surfaces can vary considerably.

IV. STRUCTURES OF THE RECEPTOR MOLECULES

The atomic structure of the amino-terminal two domains, D1D2, of ICAM-1 has been determined in independent crystallographic studies (Casasnovas *et al.,* 1998; Kolatkar *et al.,* 1999) (Table 1). Also, the structure of a mostly deglycosylated ICAM-1 D1D2 fragment (residues 1–185, type 2, Table 1) (Bella *et al.,* 1998) has been established. Four independent versions of the D1D2 ICAM-1 fragment in the three available crystal structures (Table 1) each contain a different elbow angle, with the variation occurring mostly in one plane. This restricted variation exists despite differences in the crystal packing environments and glycosylation properties. The lack of a spacer region between D1 and D2 permits several close interactions between the two domains and appears to be the basis for the restricted flexibility. This restriction significantly reduced the number and range of search parameters required to fit the ICAM-1 structure to the cryo-EM density maps. The structure of the PV receptor, CD155, has yet to be determined at high resolution. However, model building, based on known homologous structures, has provided some preliminary structural insights (Belnap *et al.,* 2000a; Xing *et al.,* 2000; He *et al.,* 2000).

V. RECEPTOR GLYCOSYLATION SITES GUIDE ORIENTATION DETERMINATION

Cryo-EM density maps provide direct evidence for the presence and location of carbohydrate moieties on the receptor molecules and thereby help confirm and improve the accuracy of fitting receptor molecule atomic models into the cryo-EM reconstructions. Cryo-EM reconstruction of HRV16 complexed with fully glycosylated D1D2 ICAM-1 (type 1, Table 1) and mostly unglycosylated D1D2 ICAM-1 (type 2, Table 1) was used to produce a difference map (Fig. 5) that identified the three deglycosylated sites and thus confirmed the fit of the D1D2 ICAM-1 structure into the electron density for the virus–receptor complex.

Although only the glycosylated form of CD155 was used to study PV1–CD155 complexes by cryo-EM reconstruction, He *et al.* (2000) used a model of the unglycosylated CD155 fitted to the cryo-EM density to produce a difference map (Fig. 4) to confirm fit of the model to the cryo-EM map (He *et al.,* 2000). Belnap *et al.* (2000a) used the "bumps" on the receptor molecules protruding from the viral surface to identify the glycosylation sites.

VI. THE FOOTPRINT OF THE RECEPTOR ON THE VIRAL SURFACE

The tip of ICAM-1 D1 and the canyon wall and floor of HRV16 and HRV14 exhibit extensive shape and charge complementarity (Kolatkar *et al.,* 1999). HRVs bind to

ICAM-1, but not to other homologous molecules, such as ICAM-2 or ICAM-3. This specificity has been rationalized by the crystallographic and sequence analysis of the BC, DE, and FG loops in domain D1, which differ in sequence and conformation (Bella *et al.,* 1998). Minor-group HRVs, which do not bind ICAM-1, are not obviously phylogenetically or structurally distinct from the major-group HRVs. Furthermore, HRV14, a major-group serotype, is more distantly related to another major-group serotype, HRV16, than to the minor-group HRV1A and HRV2 serotypes (whose structures are known). Nevertheless, the residues of HRV2 corresponding to the ICAM-1 footprint on HRV14 or HRV16 lack the charge complementarity observed for the major-group HRVs (J. Bella, N. Verdaguer, I. Fita, and M. G. Rossmann, in preparation).

The viral and receptor surfaces involved in the interface between CD155 and PV1 are in excellent agreement with mutational data (Bernhardt *et al.,* 1994; Colston and Racaniello, 1994; Morrison *et al.,* 1994; Harber *et al.,* 1995; Liao and Racaniello, 1997) and, hence, validate the accuracy of the model-building study (Belnap *et al.,* 2000a; He *et al.,* 2000). 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 the floor of the PV canyon. Since the D1 domain of CD155 adopts a more tangential orientation relative to the virus surface than ICAM-1, much of its C,C′,C″ face makes additional, extensive contact with the PV surface. Utilization of the C,C′,C″ face by CD155 for interaction with its viral ligand is similar in this respect to CD4 with HIV (Kwong *et al.,* 1998). The CD155 footprint occupies about 1300 Å^2 of the PV surface, whereas the ICAM-1 footprint on HRVs covers only 900 Å^2 (Kolatkar *et al.,* 1999). The larger CD155 footprint is a consequence of the contacts made between the additional C,C′,C″ surface of CD155 and the viral surface.

VII. POSSIBLE MECHANISMS FOR RECEPTOR-INDUCED VIRAL UNCOATING

Substantial evidence [see Section II on canyon hypothesis (Kolatkar *et al.,* 1999)] 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 within the canyon and thereby possibly compete out the lipid moiety in the VP1 pocket (Rossmann, 1994) (Fig. 6). Loss of "pocket factor" then presumably leads to virus destabilization and progressive disassembly and release of the genomic RNA. CD155 binding may follow a similar pathway, as evidenced in EM by the substantial loss of particles upon incubation of PV with soluble CD155 (He *et al.,* 2000). It has been speculated (Kolatkar *et al.,* 1999) that the natural breathing of picornaviruses (Lewis *et al.,* 1998) might facilitate receptor binding to both the north and south walls of the canyon and, thus, maintain a channel along

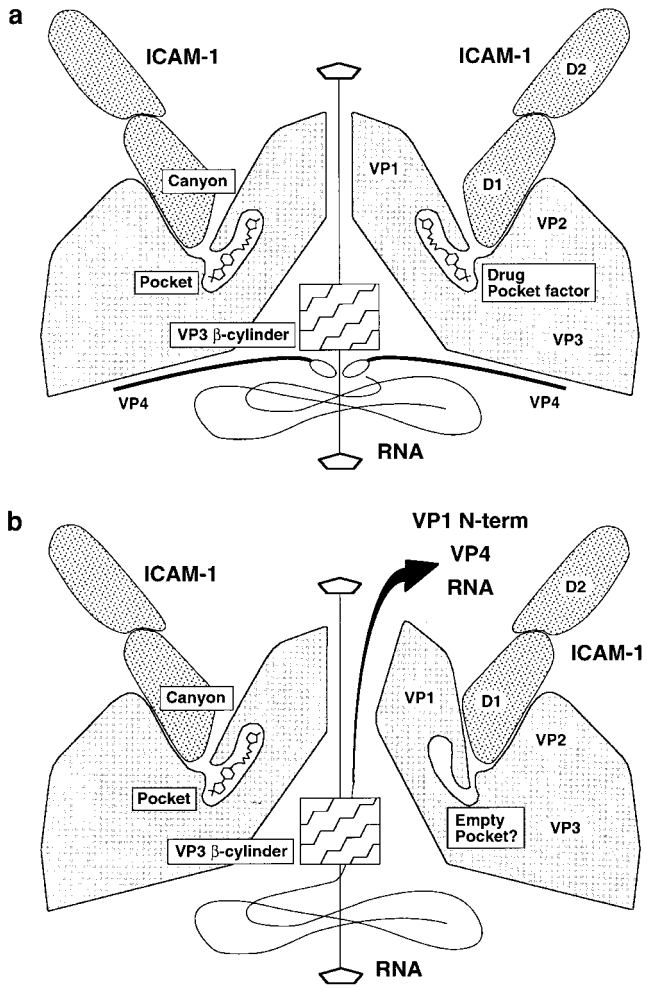

FIG. 6. Schematic representation of a proposed two-step binding mechanism between ICAM-1 and major-group HRVs. ICAM-1 is represented only as a two-domain fragment. (a) The first (observed) step corresponds to the cryo-EM reconstructions of HRV–ICAM-1 fragments in which ICAM-1 binds primarily to the floor and south wall of the canyon; (b) the second (hypothesized) step involves a conformational change in the virus surface, shown only on the right-hand side of the diagram. Probably both walls and the floor of the canyon bind to domain D1 of ICAM-1 and, in so doing, open up the fivefold channel. This requires conformational flexibility of VP1, which forms a large part of both the north and south walls of the canyon, and probably also an empty hydrophobic pocket in VP1. Opening of the pentamer vertex, induced by the binding of one or more ICAM-1 molecules, may facilitate externalization of VP4 and other internal viral components, including RNA. [Reprinted with permission from Kolatkar *et al.* (1999) Copyright European Molecular Biology Organization.]

the fivefold axis to permit the externalization of VP4, the amino end of VP1, and, eventually, the RNA. For PV, the receptor already appears to contact both walls of the canyon in the initial recognition event. The presence of CD155, therefore, may simply prevent natural breathing in PV and keep pores open as the receptor binds deeper into the canyon.

The markedly different mode of interaction of CD155 with PV1, of ICAM-1 with HRVs, and of ICAM-1 with

coxsackievirus A21 might seem surprising. Nevertheless, these receptors share several common features: they all bind into the picornavirus canyon, initiate uncoating, and are long, thin molecules that extend far from the cell surface. The similar location of binding in the canyon suggests that it is the site itself that is important, not the orientation that the bound receptor adopts. The common binding site is required to hide a part of the site from neutralizing antibodies (Rossmann *et al.,* 1985) and to regulate virus stability by competition between the binding of receptor and the lipid-like pocket factor in VP1 (Oliveira, 1993; Rossmann, 1994). The apparent need to utilize a receptor molecule that is long and extends far from the cell surface may indicate a requirement for the virus to bind to molecules that, by virtue of Brownian motion, are mobile and, hence, promote binding of multiple receptors to unoccupied binding sites on the virus, thereby facilitating cell entry and uncoating.

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