STRUCTURE OF A HUMAN RHINOVIRUS COMPLEXED WITH ITS RECEPTOR MOLECULE

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INTRODUCTION

Human rhinoviruses are one of the major causes of the common cold. They, like other picornaviruses, are icosahedral assemblies of 60 protomers that envelope a single, positive-sense strand of RNA. Each protomer consists of four polypeptides, VP1 - VP4. The three external viral proteins (VP1 - VP3) each have an approximate molecular weight of 30,000 and a similar folding topology (Rossmann *et al.*, 1985; Hogle *et al.*, 1985). The external viral radius is ~150 Å and the total molecular weight is roughly 8.5×10^6. A surface depression, or canyon, that is about 12 Å deep and 12 - 15 Å wide, encircles each pentagonal vertex (Fig. 1C). Residues lining the canyon are more conserved than other surface residues among rhinovirus serotypes[3]. The most variable surface residues are at the sites of attachment of neutralizing antibodies (Rossmann *et al.*, 1985; Sherry and Ruecker, 1985; Sherry *et al.*, 1986). It has been proposed that the cellular receptor molecule recognized by the virus binds to conserved residues in the canyon, thus escaping neutralization by host antibodies that are too big to penetrate into that region. This hypothesis (Rossmann *et al.*, 1985; Rossmann, 1989) is supported by site-directed mutagenesis of residues lining the canyon which alters the ability of the virus to attach to HeLa cell membranes (Colonno *et al.*, 1988).

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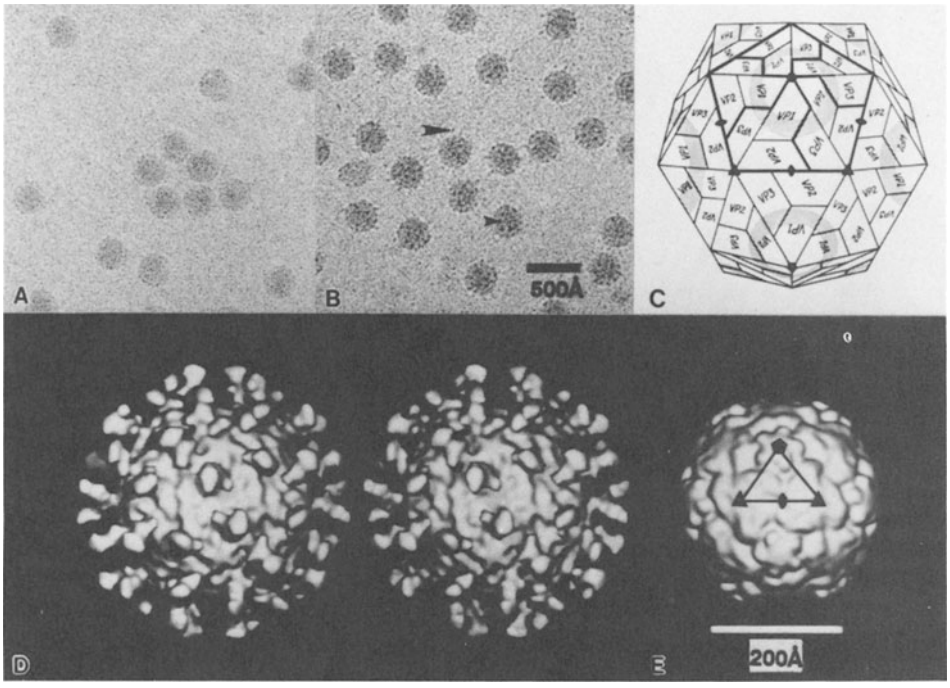

Figure 1. Cryoelectron microscopy of HRV16 particles and their complex with D1D2. (A) Native HRV16. (B) HRV16:D1D2 complex. D1D2 molecules (the two amino terminal domains of ICAM-1) are seen edge-on at the periphery of the virions (large arrow), or end-on in projection (small arrow). Cryoelectron microscopy was performed essentially as described by Cheng *et al.* (1992) with images recorded at a nominal magnification of 49,000X and with an electron dose of ~20e^-/Å^2. (C) Schematic diagram of HRV showing the icosahedral symmetry, subunit organization and canyon (shaded). Thick lines encircle five protomers of VP1, VP2, and VP3. The fourth viral protein, VP4, is inside the capsid. (D) Stereoview of the reconstruction of the HRV16:D1D2 complex, viewed along an icosahedral twofold axis in approximately the same orientation as in (C). Sixty D1D2 molecules are bound to symmetry-equivalent position at the twelve canyon regions on the virion. The reconstruction was modified to correct for defocus and amplitude contrast effects present in the original micrographs (R.H. Cheng, manuscript submitted). (E) Shaded-surface view of HRV14, computed from the atomic structure (Rossmann *et al.*, 1985), truncated to 20 Å resolution. The triangular outline of one icosahedral asymmetric unit corresponding to that in (C) is indicated.

Also, conformational changes in the floor of the canyon, produced by certain antiviral agents that bind into a pocket beneath the canyon floor, inhibit viral attachment to cellular membranes (Pevear *et al.*, 1985). Conservation of the viral attachment site inside a surface depression has been observed for Mengo (Kim *et al.*, 1990) and influenza virus (Weis *et al.*, 1988; Colman *et al.*, 1983).

There are well over 100 rhinovirus serotypes, which can be divided into roughly two groups according to the cellular receptor they recognize (Abraham and Colonno, 1984; Uncapher *et al.*, 1991). The structures of human rhinovirus 14 (HRV14) (Rossmann *et al.*, 1985), which belongs to the major group of serotypes, and of HRV1A (Kim *et al.*, 1989), which belongs to

the minor group of serotypes, have been determined. The structure of HRV16, another major group rhinovirus, is currently being investigated (M.A. Oliveira, R. R. Rueckert & M. G. Rossmann, unpublished results). There are at least 78 serotypes (Tomassini *et al.*, 1989) that bind to intercellular adhesion molecule-1 (ICAM-1), the major group rhinovirus receptor (Greve *et al.*, 1989; Staunton *et al.*, 1989). The ICAM-1 has five immunoglobulin-like domains (D1 to D5 numbered sequentially from the amino end), a transmembrane portion, and a small cytoplasmic domain (Simmons *et al.*, 1988; Staunton *et al.*, 1988). Domains D2, D3 and D4 are glycosylated. Unlike immunoglobulins, ICAM-1 appears to be monomeric (Staunton *et al.*, 1989). Mutational analysis of ICAM-1 has shown that domain D1 contains the primary binding site for rhinoviruses as well as the binding site for its natural ligand, lymphocyte function-associated antigen-1 (LFA-1) (Staunton *et al.*, 1990; Lineberger *et al.*, 1990). Other surface antigens within the immunoglobulin superfamily that are utilized by viruses as receptors include CD4 for human immunodeficiency virus-1 (Robey and Axel, 1990; Maddon *et al.*, 1986), the poliovirus receptor (Mendelsohn *et al.*, 1989), and the mouse coronavirus receptor (Williams *et al.*, 1991). As in ICAM-1, domain D1 of the poliovirus receptor (Koike *et al.*, 1991; Freistadt and Racaniello, 1991) and of CD4 (Arthos *et al.*, 1989) is the primary receptor-virus binding site. The structures of the two amino-terminal domains of CD4 have been determined to atomic resolution (Wang *et al.*, 1990; Ryu *et al.*, 1990). Truncated proteins corresponding to the two amino-terminal domains of ICAM-1 (tICAM-1(185)) as well as the intact extracellular portion of ICAM-1 (tICAM-1(453) or domains D1 to D5) have been expressed in CHO cells (Greve *et al.*, 1991). The desialated form of tICAM-1(185), which will be referred to hereafter as molecule D1D2, has recently been crystallized (Kolatkar *et al.*, 1992).

The attachment of rhinovirus to the receptor molecule at the cell surface is only the first step of virus uncoating. Subsequent to binding receptor, virus is apparently internalized by receptor-mediated endocytosis and enters the endosomal compartment. Productive rhinovirus uncoating and infection requires an intracellular low pH step (Madshus *et al.*, 1984). *In vitro*, low pH treatment will convert rhinovirus to both 135S (missing VP4) and 80S (missing VP4 and RNA) subviral particles (Korant *et al.*, 1975). A number of studies have shown that poliovirus can be conformationally altered to a 135S form upon interaction with its receptor (Gromeier and Wetz, 1990), and rhinovirus can be converted to an 80S empty capsid by incubation in the presence of soluble ICAM-1 (Greve *et al.*, 1991). Thus, both virus-receptor binding and low pH (presumably in the endosomal compartment) appear to play active roles in the controlled disassembly of virus during uncoating, although the relative contributions of these two factors and their temporal relationship *in vivo* are unclear.

A model of the amino-terminal domain D1 of ICAM-1, based on its homology to known structures of the constant domains of immunoglobulins, was reported by Giranda *et al.* (1990) Guided by mutational studies of HRV14 and ICAM-1, they were able to fit this model into the known canyon structure of HRV14. We have utilized cryoelectron microscopy and image analysis techniques to calculate a three-dimensional reconstruction of the complex of HRV16 and D1D2 to ~28 Å resolution. The reconstruction clearly shows that the receptor binds into the canyon of rhinovirus as predicted (Rossmann *et al.*, 1985; Rossmann, 1989). In addition, we use the known structures of HRV14 and CD4 and the predicted structure of D1 of ICAM-1 to identify atomic interactions.

STRUCTURE OF THE VIRUS: RECEPTOR MODEL

Initial attempts at observing complexes of ICAM-1 with HRV14 failed due to the instability of HRV14 in the presence of bound receptor (Greve *et al.*, 1991). Electron micrographs of such specimens revealed severely disrupted particles in a background of protein. However, analysis of several rhinovirus serotypes indicated that HRV14 rapidly uncoated to 80S empty capsids in the presence of soluble ICAM-1, whereas HRV3 and HRV16 formed stable virus-receptor complexes under the same conditions (H. Hoover-Litty & J. M. Greeve, in preparation). HRV16 complexes with the D1D2 molecule or with the complete D1 to D5 extracellular fragment were both used in the investigation. We present here only the results obtained on the HRV16:D1D2 complex.

Unstained vitrified HRV16 and HRV16:D1D2 (Figs. 1A, 1B) have very low, inherent contrast, and the recorded micrographs were very noisy because of the required levels of defocus (~0.8μm) and irradiation (~20e^-/Å^2). The only readily visible details on the complexes (Fig. 1B) are the D1D2 molecules that are either seen edge-on at the periphery of the virions or end-on in projection. Forty-four images of the complex were combined to compute a three-dimensional reconstruction (Fig. 1D) with an effective resolution of ~28Å (ref. 40). Although each of the images could be aligned with respect to a consistent choice of enantiomorph, in the absence of additional information there was no way to determine the absolute hand of the reconstruction. However, the asymmetric distribution of density features about the three- and fivefold axes in both the reconstruction and the known HRV14 structure (Fig, 1E) was clearly evident and unambiguously established that the reconstruction had been computed with the correct hand. The excellent correspondence between the asymmetric features provided added confirmation that the reconstruction was accurate. Furthermore, the correlation coefficient between the EM and X-ray maps for densities between radii of 125 to 150 Å was 0.67 for the correct hand versus 0.53 for the opposite hand. The density value of the D1D2 feature in the reconstruction was roughly the same as the density of the virion capsid, thus indicating that the D1D2 molecules nearly saturated the 60 available sites on the virion. Each D1D2 molecule has an approximate dumbbell shape, consistent with the presence of a two-domain structure.

A difference map between the EM model and 20 Å HRV14 model was computed after the radial scale of the EM map was adjusted to correct for magnification errors and after the EM data were compensated to correct for the effects of the microscope contrast transfer function (Toyoshima and Unwin, 1988) (R. H. Cheng, manuscript submitted). The difference map showed that the D1D2 molecule binds to the central portion of the canyon in a manner roughly as predicted by Giranda *et al.* (1990), confirming the predictions inherent in the canyon hypothesis (Rossmann, 1989). The D1D2 molecule is closely associated with the "southern" (see Fig. 2) wall and rim of the canyon, extending 10 Å to 12 Å into the canyon. The binding site is near the center of the triangle formed between a fivelold and two adjacent threefold axes (Fig. 2). The ICAM fragment is oriented roughly perpendicular to the viral surface and extends to a radius of ~205 Å. Its total length is ~75 Å as measured in the difference map. Studies with interspecies chimeras and site-directed mutagenesis of ICAM-1 aimed at identifying the regions necessary for rhinovirus binding indicated that only the first domain is essential, and that the residues most involved in virus binding were concentrated on the outside end of domain D1 (Staunton *et al.*, 1990; McClelland *et al.*, 1991). However, it has proven difficult to produce an active form of domain D1 solution, domains D1 + D2 being the minimal soluble

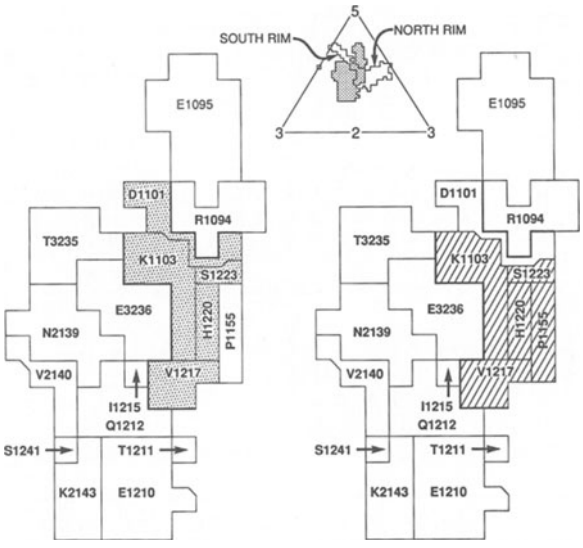

Figure 2. Top: View of the icosahedral asymmetric unit bounded by adjacent five- and threefold axes, outlining residues on the viral surface. Shown are the limits of the canyon, arbitrarily demarcated by a 138 Å radial distance from the viral center[3], and the ICAM-1 footprint (stippled). Left and right: Enlarged view of the residues in the ICAM-1 footprint showing (right) the residues which, when mutated, affect viral attachment (Colonno *et al.*, 1988), and (left) the residues altered in structure by the binding of antiviral compounds that inhibit attachment and uncoating (Smith *et al.*, 1986).

virus-binding species (Greve *et al.*, 1991). The inability to produce domain D1 in isolation and the sequence alignment between ICAM-1 and CD4 suggested that domains D1 and D2 of ICAM-1 are intimately associated through a common, extended β-strand as is seen in the structure of CD4 (Wang *et al.*, 1990; Ryu *et al.*, 1990). Thus, it seemed reasonable to use the known structures of CD4 for fitting the reconstructed density map, although there was slightly too little density for domain D1 and too much density for D2. A better assessment of the fit of domain D1 to the density was obtained by taking the predicted D1 structure of ICAM-1, including all side chains, and superimposing it onto the fitted C_α backbone of CD4. The resultant sequence alignment is shown in Figure 3. One major difference is that although domain D1 of CD4 resembles a variable, immunoglobulin-like domain with extra β-strands, the ICAM-1 prediction is based on a more likely analogy to an immunoglobulin constant domain. This gives domain D1 of ICAM-1 a sleeker appearance, consistent with the observed difference density. The extra density in D2 compared to D2 of CD4 is probably due to the associated carbohydrate groups.

The atomic structure of HRV14 closely matched the reconstructed density that was not occupied by the D1D2 fragment. The only exception occurred in the BC loop of VP1 of HRV14 (see Fig. 4), which extended about 3Å outside the reconstructed density on the "northern" rim of the canyon. However, this region of the polypeptide chain is a highly variable structure and is the site of one of the two largest conformational differences between HRV14 and HRV1A
(Kim *et al.*, 1989). Furthermore, it is also the site of major differences in the structures of homologous poliovirus serotypes 1 and 3 (Filman *et al.*, 1989).

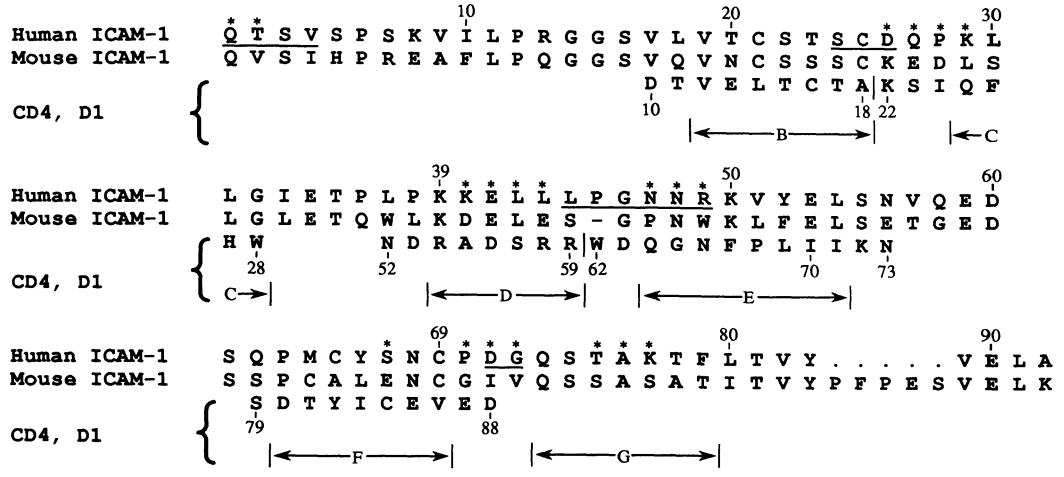

Figure 3. Sequence alignment of domain D1 for human and mouse ICAM-1 (Siu *et al.*, 1989). Also shown is the alignment of ICAM-1 with CD4 based on the predicted structure of the former (Giranda *et al.*, 1990) and the known structure of the latter (Wang *et al.*, 1990; Ryu *et al.*, 1990). Residues marked with asterisks indicate positions implicated in virus binding by site-directed mutagenesis of ICAM-1. The criteria for involvement in virus binding is a reduction of approximately 90% or more in virus binding to ICAM-1 mutants in the absence of evidence for gross structural defects (Staunton *et al.*, 1990; McClelland *et al.*, 1991; Register *et al.*, 1991). Positions identified by Staunton *et al.* (1990) are: 1 - 2, 26 - 29 and 46 - 48; positions identified by McClelland *et al.* (1991) are: 40 - 43, 70 - 72 and 75 - 77; positions identified by Register *et al.* (1991) are: 26 - 30, 67 and 70. An inconsistency was observed at residue 30 (an effect was seen by Register *et al.* (1991) but not by McClelland *et al.* (1991) and is therefore not indicated. Residues of human ICAM-1 that are likely to approach atoms of HRV14 to within 4.5 Å are underlined; based on fitting the CD4 structure into the electron density and then superimposing the predicted ICAM-1, D1 structure (derived from its homology to an immunoglobulin constant domain (Giranda *et al.*, 1990) onto CD4-D1. β-sheet regions in CD4 are marked B, C, D, E and F.

The BC loop has been used extensively for generating poliovirus chimera, permitting the alteration of one serotype to another (Minor *et al.*, 1991; Altermeyer *et al.*, 1991). In the absence of the HRV16 sequence or structure, it is uncertain how far the BC loop of HRV16 would extend towards the ICAM density.

MUTATION DATA

Colonno *et al.* (1988) showed that HRV14 residues H1220*, K1103, P1155, and S1223 all affect the binding of the virus to cellular membranes. Changing P1155 to glycine resulted in enhanced binding, while other alterations of these residues were almost neutral or reduced the binding ability. All these residues are part of the canyon floor and lie centrally within the footprint of the D1D2 molecule binding site (Fig. 2).

* The first digit of the residue identification signifies the viral protein, while the last three digits give the amino acid sequence number within the protein.

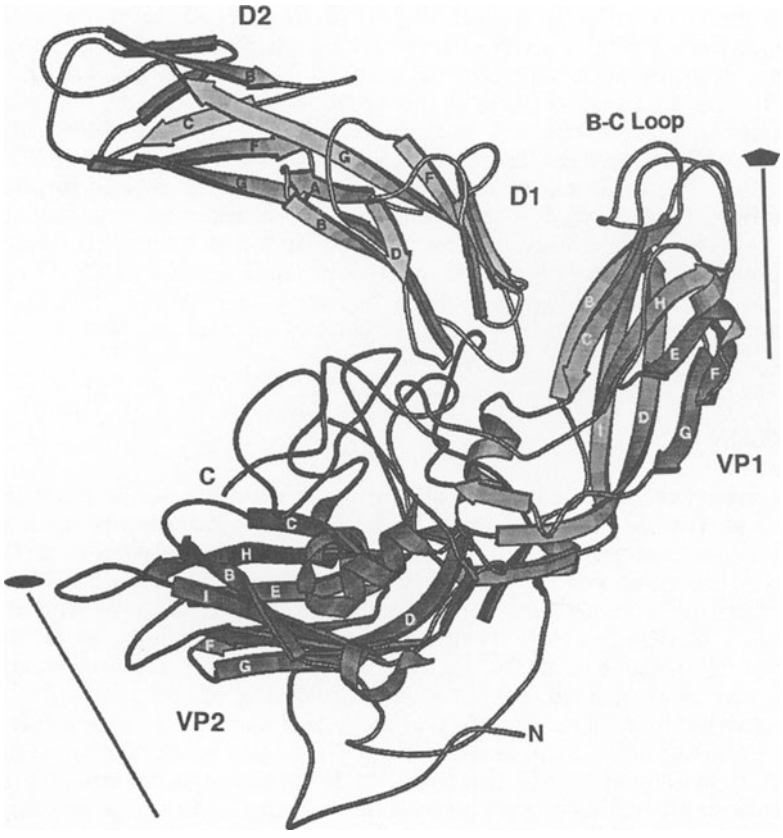

Figure 4. A diagrammatic drawing (Kraulis, 1992) showing the CD4 structure bound to HRV16 as seen in the difference density. Secondary structural elements of the CD4 fragment and HRV14 (homologous structures used to represent ICAM-1 and HRV16, respectively) are identified by the standard nomenclature. The N and C termini of VP1 are also marked.

Certain antiviral agents to rhino-and enteroviruses (Diana *et al.*, 1985) inhibit uncoating and attachment. These agents bind to a pocket beneath the canyon and, in HRV14, significantly alter the structure of the canyon floor (Smith *et al.*, 1986; Badger *et al.*, 1988). These conformational changes inhibitviral attachment (Pevear *et al.*, 1989; Heinz *et al.*, 1989) and are now shown to be exactly at the site of ICAM-1 attachment (Fig. 3).

Heinz *et al.* (1989) have examined a series of escape mutants to the antiviral uncoating inhibitors. Mutants selected in high drug concentrations have larger residues in the binding pocket that inhibit drug entry (Heinz *et al.*, 1989; Badger *et al.*, 1989). However, in lower drug concentrations, resistant mutations are found near the surface residues of the canyon. These mutations (N1100→S, N1105→S, V1153→I, N1219→S, S1223→G) permit binding of drugs into the pocket which induce the usual conformational changes, but do not inhibit viral attachment. The mutated residues are within the region of conformational changes and, hence, might allow some additional flexibility in the floor of the canyon and receptor attachment.

The parts of the predicted ICAM-1 structure that make contact with HRV14 are the amino terminal four residues and loops BC (residues 24 - 26), DE (residues 45 - 49) and FG (residues 71 - 72; see Figs. 3 and 4 for

nomenclature). Staunton *et al.* (1990), McClelland *et al.* (1991) and Register *et al.* (1991) have examined the effects of a number of site-directed mutations and mouse-human substitutions in domain D1 of ICAM-1 on rhinovirus binding. Based on these reports seven regions in D1 (Fig. 3), corresponding to the N-terminus (residues 1 - 2), loop BC (residues 26 - 29), strand D (residue 40, 43), loop DE (residues 46 - 48), strand F (residue 67), FG (residues 70 - 72), and the G strand (residues 75 - 77), have been implicated in virus binding. There is correspondence to, or significant overlap between, the four regions of ICAM-1 seen here to be in contact with rhinovirus and four of the seven regions identified by site-directed mutagenesis. Thus, there appears to be reasonable agreement between the mutational studies of ICAM-1 and the observed virus-receptor contacts of the complex.

DISCUSSION

The structure of a complex of Simian rotavirus with a neutralizing antibody Fab fragment was studied by cryoelectron microscopy in a manner similar to that reported here (Prassad *et al.*, 1990), however neither the structure of the virus nor the antibody was known in atomic detail. Recently, the structure of a complex of cowpea mosaic virus (CPMV) and a bound monoclonal antibody Fab fragment was determined with electron microscopy (Wang *et al.*, 1992). In that case, an atomic resolution structure of CPMV was known, permitting the determination of the antibody footprint on the viral surface. Even more recently, the structure of a neutralating antibody Fab fragment, complexed with HRV14, has been determined (Smith *et al.*, 1992), which suggests the mode of bivalent attachment required for neutralization. Here these cryoelectron microscopy techniques are applied to a virus-receptor complex.

Weis *et al.* (1988) and Sauter *et al.* (1992) have explored the interaction of a carbohydrate moiety on the surface of erythrocytes to which influenza virus can attach. We describe here first structure of a virus-receptor complex in which the receptor is a membrane-bound glycoprotein molecule that is used by a virus for recognition of a specific host tissue for attachment and subsequent entry. This receptor molecule belongs to the immunoglobulin superfamily, a class of molecule frequently employed on cell surfaces for the recognition of other molecules (or recognized by viruses) that are subsequently transferred across the membrane. Although the structure of CD4 is known (Wang *et al.*, 1990; Ryu *et al.*, 1990), the structure of the HIV-CD4 complex is not. Mutational studies suggest that the structure recognized by HIV is a ridge made up of β-strands C" and D. Whereas strand C" probably does not exist in domain D1 of ICAM-1 (it is characteristic of immunoglobulin variable type domains), strand D in the D1D2 fragment of ICAM-1 does make particularly close contact, as does also the amino-terminal βA strand. The structure of a complex of human growth factor (hGF) and its immunoglobulin-like receptor has been determined (de Vos *et al.*, 1992). In this complex the primary contact regions between the two receptor molecules and hGF are the AB and EF loops and the βG strand, on the opposite end of the immunoglobulin molecule as those identified for making contact with the rhinovirus canyon.

The structure of a virus-receptor complex might give some indication of the subsequent steps which permit the virus to enter and infect the host cell. Furthermore, since the general nature of the complex described here had been predicted on the basis of the strategy used by HRV to hide its receptor attachment site, perhaps many other viruses use a similar strategy.

Poliovirus is clearly homologous to HRV, and both poliovirus (Mendelsohn *et al.*, 1989) and the major rhinovirus group use an immunoglobulin-like molecule as receptor. Thus, it would be expected that the poliovirus receptor binds into the poliovirus canyon in a manner similar to that of the complex formed for rhinoviruses (Freistadt and Racaniello, 1991). The structure of a mouse-adapted chimera of human poliovirus 2 has been determined (Yeates *et al.*, 1991). The major structural change occurs in the chimera in the BC loop, not in the canyon floor. In this instance, therefore, the BC loop might modulate the virus-receptor interaction.

The determination of residues involved in receptor binding should make it possible to ascertain the origin of specificity of the major rhinovirus serotypes for ICAM-1. Comparison of the amino acid sequences of six rhinoviruses belonging to the major receptor group against four of the minor receptor group did not reveal any clear differentiation. However, structural and binding investigations tentatively suggest that major rhinoviruses do not bind a lipid or fatty acid (Kim *et al.*, 1989; Filman *et al.*, 1989; Kim *et al.*, 1992) within the pocket utilized by the attachment-uncoating inhibitors, in contrast to the minor receptor group of rhinoviruses. Therefore, specificity for the minor receptor may reside in the virus' tendency to bind tightly a cellular fatty acid (Kim *et al.*, 1989; Kim *et al.*, 1992) and thus alter the shape of the canyon floor, rather than the identity of the canyon surface residues themselves. Nevertheless, kwowledge of the virus-receptor interaction will illuminate various strategies currently being developed to interfere with early stages of rhinoviral and other viral infections (Greve *et al.*, 1991; McKinlay *et al.*, 1992; Marlin *et al.*, 1990).

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REFERENCES

Abraham, G. and Colonno, R.J., 1984, Many rhinovirus serotypes share the same cellular receptor, J Virol. 51: 340-345.

Altermeyer, R., Murdin, A.D., Harber, J.J. and Wimmer, E., 1991, Construction and characterization of a poliovirus/rhinovirus antigenic hybrid, Virology. 184: 636-644.

Arthos, J., Deen, K.C., Chaikin, M.A., Fornwald, J.A., Sathe, G., Sattentau, Q.J., Clapham, P.R., Weiss, R.A., McDougal, J.S., Pietropaolo, C., Axel, R., Truneh, A., Maddon, P.J. and Sweet, R.W., 1989, Identification of the residues in human CD4 critical for the binding of HIV, Cell. 57:469-481.

Badger, J., Krishnaswamy, S., Kremer, M.J., Oliveira, M.A., Rossmann, M.G., Heinz, B.A., Rueckert, R.R., Dutko, F.J. and McKinlay, M.A., 1989, The three-dimensional structures of drug-resistant mutants of human rhinovirus 14, J Mol Biol. 207: 163-174.

Badger, J., Minor, I., Kremer, M.J., Oliveira, M.A., Smith, T.J., Griffith, J.P., Guerin, D.M.A., Krishnaswamy, S., Luo, M., Rossmann, M.G., McKinlay, M.A., Diana,

G.D., Dutko, F.J., Fancher, M., Rueckert, R.R. and Heinz, B.A., 1988, Structural analysis of a series of antiviral agents complexed with human rhinovirus 14, Proc Natl Acad Sci USA. 85: 3304-3308.

Baker, T.S., Newcomb, W.W., Olson, N.H., Cowsert, L.M., Olson, C. and Brown, J.C., 1991, Structures of bovine and human papillomaviruses: analysis cryoelectron microscopy and three-dimensional image reconstruction, Biophys J. 60: 1445-1456.

Colman,P.M., Varghese, J.N., and Laver, W.G., 1983, Structure of the catalytic and antigenic sites in influenza virus neuraminidase, Nature. 303: 41-44.

Colonno, R.J., Condra, J.H., Mizutani, S., Callahan, P.L., Davies, M.E. and Murcko, M.A., 1988, Evidence for the direct involvement of the rhinovirus canyon in receptor binding, Proc Natl Acad Sci USA. 85:5449-5453.

Cheng, R.H., Olson, N.H. and Baker, T.S., 1992, Cauliflower mosaic virus: a 420 subunit (T = 7), multilayer structure, Virology. 186: 655-668.

Dalgleish, A.G., Beverly, P.C.L., Clapham, P.R., Crawford, D.H., Greaves, M.F. and Weiss, R.A., 1984, The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus, Nature (London). 312: 763-767.

de Vos, A.M., Ultsch, M. and Kossiakoff, A.A., 1992, Human growth hormone and extracellular domain of its receptor: crystal structure of the complex, Science. 255: 306-312.

Diana, G.D., McKinlay, M.A., Otto, M.J., Alullian, V. and Oglesby, C., 1985, [[(4,5-Dihydro-2-oxazolyl)phenoxy]alkyl]isoxazoles. Inhibitors of viral uncoating, J Med Chem. 28: 1906-1910.

Filman, D.J., Syed, R., Chow, M., Macadam, A.J., Minor, P.D. and Hogle, J.M., 1989, Structural factors that control conformational transitions and serotype specificity in type 3 poliovirus, EMBO J. 8: 1567-1579.

Freistadt, M.S. and Racaniello, V.R., 1991, Mutational analysis of the cellular receptors for poliovirus, J Virol. 65: 3873-3876.

Giranda, V.L., Chapman, M.S. and Rossmann, M.G., 1990, Modeling of the human intercellular adhesion molecule-1, the human rhinovirus major group receptor, Proteins. 7: 227-233.

Greve, J.M., Davis, G., Meyer, A.M., Forte, C.P., Yost, S.C., Marlor, C.W., Kamarck, M.E. and McClelland, A., 1989, The major human rhinovirus receptor is ICAM-1, Cell. 56: 839-847.

Greve, J.M., Forte, C.P., Marlor, C.W., Meyer, A.M., Hoover-Litty, H., Wunderlich, D. and McClelland, A., 1991, Mechanisms of receptor-mediated rhinovirus neutralization defined by two soluble forms of ICAM-1, J Virol. 65: 6015-6023.

Gromeier, M. and Wetz, K.J., 1990, Kinetics of poliovirus uncoating in HeLa cells in a nonacidic environment, J Virol. 64: 3590-3597.

Heinz, B.A., Rueckert, R.R., Shepard, D.A., Dutko, F.J., McKinlay, M.A., Fancher, M., Rossmann, M.G., Badger, J. and Smith, T.J., 1989, NGenetic and molecular analyses of spontaneous mutants of human rhinovirus 14 are resistant to an antiviral compound, J Virol. 63: 2476-2485.

Hogle, J.M., Chow, M. and Filman, D.J., 1985, Three-dimensional structure of poliovirus at 2.9 Å resolution, Science. 229: 1358-1365.

Kim, K.H., Willingmann, P., Gong, Z.X., Kremer, M.J., Chapman, M.S., Minor, I., Oliveira, M., Rossmann, M.G., Andries, K., Diana, G.D., Dutko, F.J., McKinlay, M.A. and Pevear, D.C., 1992, A Comparison of the Anti-rhinoviral Drug Binding Pocket in HRV14 and HRV1A, manuscript in preparation.

Kim, S. , Boege, U., Krishnaswamy, S., Minor, I., Smith, T.J., Luo, M., Scraba, D.G. and Rossmann, M.G., 1990, Conformational variability of a picornavirus capsid: pH-dependent structural changes of Mengo virus related to its host receptor attachment site and disassembly, Virology. 175: 176-190.

Kim, S., Smith, T.J., Chapman, M.S., Rossmann, M.G., Pevear, D.C., Dutko, F.J., Felock, P.J., Diana, G.D. and McKinlay, M.A., 1989, The crystal structure of human rhinoviruses serotype 1A (HRV1A), J Mol Biol. 210: 91-111.

Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercend, T., Gluckman, J.-C. and Montagnier, L., 1984, T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV, Nature (London). 312: 767-768.

Koike, S., Ise, I. and Nomoto, A., 1991, Functional domains of the poliovirus receptor, Proc Natl Acad Sci USA. 88: 4104-4108.

Kolatkar, P.R., Oliveira, M.A., Rossmann, M.G., Robbins, A.H., Katti, S.K., Hooer-Litty, H., Forte, C., Greve, J.M., McClelland, A. and Olson, N.H., 1992, Preliminary X-ray crystallographic analysis of intercellular adhesion molecule-1, J Mol Biol., in press.

Korant, B.D., Lonberg-Holm, K., Yin, F.H. and Noble-Harvey, J., 1975, Fractionation of biologically active and inactive populations of human rhinovirus type 2, Virology. 63: 384-394.

Kraulis, P.J.,1992, J Appl Crystallogr. 24: 946-950.

Lineberger, D.W., Graham, D.J., Tomassini, J.E. and Colonno, R.J., 1990, Antibodies that block rhinovirus attachment map to domain 1 of the major group receptor, J Virol. 64: 2582-2587.

Maddon, P.J., Dalgleish, A.G., McDougal, J.S., Clapham, P.R., Weiss, R.A. and Axel, R., 1986, The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain, Cell. 47: 333-348.

Madshus, I.H., Olsnes, S. and Sandvig, K., 1984, Different pH requirements for entry of the two picornaviruses, human rhinovirus 2 and murine encephalomyocarditis virus, Virology. 139: 346-357.

Marlin, S.D., Staunton, D.E., Springer, T.A., Stratowa, C., Sommergruber, W. and Merluzzi, V.J., 1990, A soluble form of intercellular adhesion molecule-1 inhibits rhinovirus infection, Nature (London). 344: 70-72.

McClelland, A., deBear, J., Yost, S.C., Meyer, A.M., Marlor, C.W. and Greve, J.M., 1991, Identification of monoclonal antibody epitopes and critical residues for rhinovirus binding in domain 1 of intercellular adhesion molecule 1, Proc Natl Acad Sci USA. 88: 7993-7997.

McKinlay, M.A., Pevear, D.C. and Rossmann, M.G., 1992, Treatment of the picornavirus common cold by inhibitors of viral uncoating and attachment, Ann Rev Microbiol. manuscript in press.

Mendelsohn, C.L., Wimmerm, E. and Racaniello, V.R., 1989, Cellular receptors for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily, Cell. 56: 855-865.

Minor, P.D., Ferguson, M., Katrak, K., Wood, D., John, A., Howlett, J., Dunn, G., Burke, K. and Almond, J.W., 1991, Antigenic structure of chimeras of type 1 and type 3 polioviruses involving antigenic sites 2, 3 and 4, J Gen Virol. 72: 2475-2481.

Pevear, D.C., Fancher, M.J., Felock, P.J., Rossmann, M.G., Miller, M.S., Diana, G., Treasurywala, A.M., McKinlay, M.A. and Dutko, F.J., 1989, Conformation change in the floor of the human rhinovirus canyon blocks adsorption to HeLa cell receptors, J Virol. 63:2002-2007.

Prasad, B.V.V., Brurns, J.W., Marietta, E., Estes, M.K. and Chiu, W., 1990, Localization of VP4 neutralization sites in rotavirus by three-dimensional cryo-electron microscopy, Nature (London). 343: 476-479.

Register, R.B., Uncapher, C.R., Naylor, A.M., Lineberger, D.W. and Colonno, R.J., 1991, Human-murine chimeras of ICAM-1 identify amino acid residues critical for rhinovirus and antibody binding, J Virol. 65: 6589-6596.

Robey, E. and Axel, R., 1990, CD4: collaborator in immune recognition and HIV infection, Cell. 60: 697-700.

Rossmann, M.G., 1989, Hiding the host cell receptor attachment site on a viral surface from immune surveillance, J Biol Chem. 263: 14587-14590.

Rossmann, M.G. and Palmenberg, A.C., 1988, Conservation of the putative receptor attachment site in picornaviruses, Virology. 164: 373-382.

Rossmann, M.G., Arnold, E., Erickson, J.W., Frankenberger, E.A., Griffith, J.P., Hecht, H.J., Johnson, J.E., Kamer, G., Luo, M., Ruekert, A.G., Sherry, B. and Vriend, G., 1985, Structure of a human common cold virus and functional relationship to other picornaviruses, Nature (London). 317:145-153.

Ryu, S.E., Kwong, P.D., Truneh, A., Porter, T.G., Arthos, J., Rosenberg, M., Dai, X., Xuong, N., Axel, R., Sweet, R.W. and Hendrickson, W.A., 1990, Crystal structure of an HIV-binding recomginant fragment of human CD4, Nature (London). 348: 419-426.

Sauter, N.K., Glick, G.D., Crowther, R.L., Park, S.J., Eisen, M.B., Skehel, J.J., Knowles, J.R. and Wiley, D.C., 1992, Crystallographic detection of a second ligand binding site in influenza virus hemagglutinin, Proc Natl Acad Sci USA. 89: 324-328.

Sherry, B. and Rueckert, R., 1985, Evidence for at least two dominant neutralization antigens on human rhinovirus 14, J Virol. 53: 137-143.

Sherry, B., Mosser, A.G., Colonno, R.J. and Rueckert, R.R., 1986, Use of monoclonal antibodies to identify four neutralization immunogens on a common cold picornavirus, human rhinovirus 14, J Virol. 57: 246-257.

Simmons, D., Makgoba, M.W. and Seed, B., 1988, ICAM, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM, Nature (London). 331: 624-627.

Siu, G., Hedrick, S.M. and Bian, A.A., 1989, Isolation of the murine intercellular adhesion molecule 1 (ICAM-1) gene, J Immunol. 143: 3813-3820.

Smith, T.J., Kremer, M.J., Luo, M., Vriend, G., Arnold, E., Kamer, G., Rossmann, M.G., McKinlay, M.A., Diana, G.D. and Otto, M.J.,1986, The site of attachment in human rhinovirus 14 for antiviral agents that inhibit uncoating, Science. 233: 1286-1293.

Smith, T.J., Olson, N.H., Cheng, R.H., Chase, E., Lee, W.M., Leippe, D., Mosser, A., Rueckert, R.R. and Baker, T.S., 1992, Structure of human rhinovirus complexed with Fab fragments from a neutralizing antibody, Nature (London). Manuscript submitted for publication.

Staunton, D.E., Dustin, M.L., Erickson, H.P. and Springer, T.A., 1990, The arrangement of the immunoglobulin-like domains of ICAM-1 and the binding site for LFA-1 and rhinovirus, Cell, 61: 243-254.

Staunton, D.E., Marlin, S.D., Stratowa, C., Dustin, M.L., and Springer, T.A., 1988, Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families, Cell. 52: 925-933.

Staunton, D.E., Merluzzi, V.J., Rothlein, R., Barton, R., Marlin, S.D. and Springer, T.A., 1989, A cell adhesion molecule, ICAM-1, is the major suface receptor for rhinoviruses, Cell. 56: 849-853.

Tomassini, J.E., Maxson, T.R. and Colonno, R.J., 1989, Biochemical characterization of a glycoprotein required for rhinovirus attachment, J Biol Chem. 264:1656-1662.

Toyoshima, C. and Unwin, N., 1988, Contrast transfer for frozen-hydrated specimens: determination from pairs of defocused images, Ultramicroscopy. 25: 279-282.

Uncapher, C.R., DeWitt, C.M. and Colonno, R.J., 1991, The major and minor group receptor families contain all but one human rhinovirus serotype, Virology. 180: 814-817.

Wang, G., Porta, C., Chen, Z., Baker, T.S. and Johnson, J.E., 1992, Identification of a Fab interaction footprint site on an icosahedral virus by cryoelectron microscopy and X-ray crystallography, Nature (London). 355: 275-278.

Wang, J., Yan, Y., Garret, T.P.J., Liu, J., Rodgers, D.W., Garlick, R.L., Tarr, G.E., Husain, Y., Reinherz, E.L. and Harrison, S.C., 1990, Atomic structure of a fragment of human CD4 containing two immunoglobulin-like domains, Nature (London). 348: 411-418.

Weis, W., Brown, J.H., Cusack, S., Paulson, J.C., Skehel, J.J. and Wiley, D.C., 1988, Structure of the influenza virus haemagglutinin complexed with its receptor, sialic acid, Nature (London), 333: 426-431.

Williams, R.K., Jiang, G.S. and Holmes, K.V., 1991, Receptor for mouse hepatitis virus is a member of the carcinoembryonic antigen family of glycoproteins, Proc Natl Acad Sci USA. 88: 5533-5536.

Yeates, T.O., Jacobson, D.H., Margin, A., Wychowki, C., Girard, M., Filman, D.J. and Hogle, J. M., 1991, Three-dimensional structure of a mouse-adapted type 2/type 1 poliovirus chimera, EMBO J. 10: 2331-2341.