Structural Insights into Calicivirus Attachment and Uncoating^{▽†}

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The *Caliciviridae* family comprises positive-sense RNA viruses of medical and veterinary significance. In humans, caliciviruses are a major cause of acute gastroenteritis, while in animals respiratory illness, conjunctivitis, stomatitis, and hemorrhagic disease are documented. Investigation of virus-host interactions is limited by a lack of culture systems for many viruses in this family. Feline calicivirus (FCV), a member of the *Vesivirus* genus, provides a tractable model, since it may be propagated in cell culture. Feline junctional adhesion molecule 1 (fJAM-1) was recently identified as a functional receptor for FCV. We have analyzed the structure of this virus-receptor complex by cryo-electron microscopy and three-dimensional image reconstruction, combined with fitting of homology modeled high-resolution coordinates. We show that domain 1 of fJAM-1 binds to the outer face of the P2 domain of the FCV capsid protein VP1, inducing conformational changes in the viral capsid. This study provides the first structural view of a native calicivirus-protein receptor complex and insights into the mechanisms of virus attachment and uncoating.

Caliciviruses are responsible for outbreaks of acute gastroenteritis in humans, as well as respiratory illness, conjunctivitis, stomatitis, and hemorrhagic disease in animals (25, 46). The family *Caliciviridae* is divided into four genera: *Norovirus*, *Sapovirus*, *Vesivirus*, and *Lagovirus* (16). Feline calicivirus (FCV) is a member of the *Vesivirus* genus and is typically associated with respiratory illness and stomatitis in cats (42). Recent outbreaks of virulent strains of FCV have, however, demonstrated the incidence of systemic disease associated with high mortality rates (~50%) (23, 35).

Caliciviruses are small positive-sense RNA containing viruses comprising a genome approximately 7.5 kb in length, encapsidated by an icosahedral capsid that is 35 to 40 nm in diameter. The viral genome contains up to four open reading frames (ORFs), the first of which encodes a polyprotein that is posttranslationally cleaved to produce several nonstructural proteins critical to virus replication. ORF2 and ORF3 encode the major capsid protein VP1 and a putative minor structural protein VP2, respectively. ORF4, the result of a +1 frameshift in the VP1 region is, as yet, of unknown function (46).

Caliciviruses have a characteristic virion morphology consisting of 90 VP1 dimers assembled into a T=3 icosahedral shell (see Fig. S1 in the supplemental material). VP1 is divided into two domains, S and P. The S domain makes up the contiguous floor of the capsid and has a β -barrel motif, similar to that seen in many plant and animal viruses. The P domain protrudes from the capsid surface, forming arch-like structures and giving the caliciviruses their distinctive morphology. The P domain is further divided into the P1 and P2 subdomains. P2, an insert in P1, is located at the outermost face of the capsom-

ere and is the site of several neutralizing epitopes. The T=3 symmetry results in two classes of capsomere. Those arranged around the fivefold axes are composed of VP1 monomers in two different quasi-equivalent environments, termed A and B. At the twofold symmetry axes, capsomeres consist of VP1 molecules in equivalent positions and are referred to as C-C dimers (9, 39, 40).

Investigations of virus-host interactions for this family of viruses are hampered by a lack of efficient culture systems, particularly for those viruses that infect humans. FCV is therefore an attractive surrogate model for less amenable viruses, since it may be propagated in vitro. Feline junctional adhesion molecule 1 (fJAM-1) was recently identified as a functional receptor for FCV. Experiments demonstrated that transfection of the fJAM-1 gene into nonpermissive cells rendered them susceptible to FCV infection, while antibodies raised against fJAM-1 prevented virus attachment and thereby infection (29). JAM-1 is a type 1 transmembrane glycoprotein thought to regulate the formation of tight junctions in epithelial and endothelial cells. It is also found on the surface of leukocytes and blood platelets. A member of the immunoglobulin (Ig)-like superfamily of proteins, JAM-1 comprises an N-terminal signal peptide, the membrane-distal D1 and the membrane-proximal D2 Ig-like domains, a C-terminal transmembrane domain, and a short cytoplasmic tail (26, 41). Domain deletion and swapping experiments have indicated that the virus-receptor interaction predominantly involves D1, and structure-guided mutagenesis has identified a number of amino acid residues in fJAM-1 that are essential for FCV infectivity (34).

FCV has also been shown to bind α 2,6-sialic acid (44), while the human noroviruses bind histo-blood group antigens (HBGA) (19, 31). The latter interaction influences host susceptibility contingent on HBGA genotype and secretor status (8, 24, 28, 30). Glycan interactions are clearly important in calicivirus tropism and pathogenesis, although their role as sole virus-receptors is less apparent.

JAM-1 is currently the only protein receptor identified for this virus family. Understanding the interaction between FCV

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and fJAM-1 therefore offers potential insights into attachment and entry in related viruses. Moreover, such understanding may lead to development of culture systems for human pathogens in the *Caliciviridae*, since there is considerable evidence that attachment and entry is the block on efficient human calicivirus growth in cell culture (2, 18).

Ig-like cellular-adhesion proteins are frequently exploited by viruses to gain entry to the cell. Rhinoviruses use ICAM-1 (6); coxsackieviruses and adenoviruses attach to CAR (the coxsackievirus and adenovirus receptor, also found in tight junctions) (38); polioviruses use a nectin-like protein PVR (the poliovirus receptor) (21); mouse hepatitis virus, a coronavirus, binds to CEACAM1 (13); and rabies virus can enter cells expressing CD56 (neural cell adhesion molecule) (47). Indeed, human JAM-1 has been identified as a receptor for reoviruses (5). Further investigation of virus attachment to Ig-like cellular-adhesion proteins is clearly warranted and may identify common structural themes.

We have conducted an investigation of the interaction between FCV and fJAM-1, using cryo-electron microscopy (cryo-EM) and icosahedral reconstruction to solve the structure of both unlabeled virus and the virus-receptor complex. Homology models of the constituent proteins in the complex were docked to the reconstructions to construct a high-resolution model of the interaction. We show that fJAM D1 binds to the outer face of the P2 domain of FCV VP1. The contact interface includes hypervariable regions of P2 previously shown to contain neutralizing epitopes, while in fJAM, we confirm the significance of amino acid residues identified in mutagenesis studies. Furthermore, we have found that fJAM binding induces conformational changes in the viral capsid that may precede genome uncoating.

MATERIALS AND METHODS

Virus culture and purification. FCV strain F9 was propagated in Crandell Reese feline kidney cells (CRFK) cells and was purified essentially as described previously (51). Virus was released from infected CRFK cells by a single freeze-thaw, and the supernatant was clarified by low-speed centrifugation ($3,000 \times g$, 15 min at 4°C) and then filtered through a $0.2\text{ }\mu\text{m}$ -pore-size filter. Virus particles were precipitated by the addition of solid polyethylene glycol (PEG 3350) and sodium chloride to 10% (wt/vol) and 0.2 M, respectively. After overnight incubation at 4°C , precipitated virus was recovered by centrifugation ($8,000 \times g$, 30 min at 4°C) and resuspended in 0.2 M boric acid buffer (pH 7.4) containing 0.5 M sodium chloride. Insoluble material was removed by low-speed centrifugation ($8,000 \times g$, 15 min at 4°C), and virus particles were partially purified by centrifugation through a 30% (wt/vol) sucrose cushion at $28,000 \times g$ for 15 h at 4°C . The virus pellet was resuspended in phosphate-buffered saline and banded in an isopycnic cesium chloride gradient (1.31 g/ml) by centrifugation using a SW55Ti rotor at 40,000 rpm for 20 h at 4°C . Virus was removed from the gradient, dialyzed against phosphate-buffered saline, and subsequently concentrated using 100-kDa-molecular-mass-cutoff centrifugal concentrator (Vivascience). The virus concentration was determined using a BCA assay (Pierce).

fJAM-1 protein expression and purification. The signal peptide and extracellular domains of fJAM-1 were amplified by reverse transcription-PCR from RNA isolated from CRFK cells. The resultant PCR product was used to generate a eukaryotic expression plasmid (pDEF:fJAM:Fc) containing the extracellular domains of fJAM-1 fused at the C terminus to the Fc domain of human IgG1. The fJAM-1 and human IgG1 Fc domains were separated by a factor Xa cleavage site to allow subsequent removal of the Fc domain. Chinese hamster ovary cells stably expressing the soluble fJAM-1:Fc fusion protein were generated, and soluble fJAM-1:Fc was purified from tissue culture supernatant by using protein A-Sepharose. The resultant fusion protein was dimeric due to the inclusion of the human IgG1 Fc domain (data not shown). Monomeric fJAM-1 was generated by factor Xa cleavage of fJAM-1:Fc, removal of factor Xa using Xa removal resin (Qiagen), and subsequent removal of the released Fc domain using protein

A-Sepharose. Monomeric fJAM-1 was subsequently dialyzed against phosphate-buffered saline, concentrated by using a centrifugal concentrator (Vivascience), and the concentration determined by a BCA assay (Pierce).

EM. FCV virions were incubated in the presence of fJAM-1 overnight at 4°C . Unlabeled and fJAM-1-labeled virions were prepared for cryo-EM by loading 5 μl onto freshly glow-discharged quantifoil holey carbon support films (R2/2; Quantifoil, Jena, Germany), blotting, and plunging into liquid nitrogen-cooled liquid ethane (1). Vitrified specimens were imaged at a low temperature in a JEOL 2200 FS cryo-microscope equipped with a Gatan 914 cryo-stage. Energy-filtered images were recorded, with a slit width of 10 eV, on a Gatan Ultrascan 4k $\times$ 4k charge-coupled device camera at a magnification of $\times 50,000$, corresponding to a pixel-size of $2.2\text{ }\text{\AA}$ in the specimen.

Image reconstruction. Totals of 90 defocus paired images of unlabeled FCV and 120 paired images of fJAM-1-labeled FCV were recorded and processed to calculate three-dimensional reconstructions. Then, 1,810 unlabeled and 1,730 labeled particles were selected, and low-frequency variations in intensity were corrected by using X3D (10). Defocus values for each micrograph were estimated by using BSOFT (20) and ranged from 0.7 to $3.2\text{ }\mu\text{m}$. Images were corrected for the effects of the contrast transfer function with the CTFMIX program, and close-to-focus and further-from-focus particle images were merged (10).

To calculate an initial reconstruction for FCV, a common-lines approach was taken using a modified version of the MRC icosahedral reconstruction suite of programs (12, 15). The polar Fourier transform method was then used to determine origin and orientation information for both FCV and JAM-1-labeled FCV data (3, 7). Finally, the reconstructions were refined by using cross-common lines (12, 15) and calculated from 1,117 unlabeled particles and 1,281 fJAM-1-labeled particles. Resolution was assessed by dividing each data set into two equal subsets, and independent reconstructions were calculated and compared using several indices of similarity, including the Fourier shell correlation and spectral signal-to-noise ratio. Reconstructions were visualized in UCSF Chimera (36) and surface contoured to show the mean plus one standard deviation of density. Spherical section images were prepared by using UCSF Chimera or icosections (James Conway, University of Pittsburgh School of Medicine, unpublished data).

Homology modeling. To create high-resolution models of FCV VP1 and fJAM-1, homology modeling was performed by using MOE v.2007.09 (Chemical Computing Group, Montreal, Quebec, Canada). The FCV sequence was modeled against San Miguel sea-lion virus (SMSV; PDB 2GH8) (9) in multimer modeling mode without C-terminal and N-terminal outgap modeling. An initial proposed partial geometry for the FCV sequence was copied from each of the template chains in the solved structure 2GH8, using all coordinates where residue identity was conserved. Otherwise, only backbone coordinates were used. Based on this initial partial geometry, Boltzmann-weighted randomized modeling (27) was used with segment searching for regions that could not be mapped onto the initial partial geometry (14). Ten models were constructed. Upon completion of segment addition, each model was energetically minimized in the AMBER99 forcefield (49). The highest-scoring intermediate model was then determined by the generalized born/volume integral methodology (26a). Ramachandran plots on the best model revealed only a small proportion of residues outside of acceptable limits. An identical approach was applied to fJAM-1 using the structure of human JAM-1 (PDB 1NBO) (41), except that only a single chain was modeled.

Fitting high-resolution coordinates. To dock high-resolution coordinates for fJAM-1, a difference map was created by subtracting the unlabeled FCV reconstruction (filtered to $18\text{-}\text{\AA}$ resolution) from the fJAM-1-labeled reconstruction. Initial manual placements were made for each molecule (bound to either A or B positions), with either D1 or D2 closest to the outer face of the capsid, using UCSF Chimera (36). This was then refined by using Situs (50), yielding correlation values that indicated that D1 was most likely the domain that predominantly bound FCV (0.35 for D1 versus 0.31 for D2 using Laplacian filtering and 0.88 versus 0.86 using standard cross-correlation).

The FCV VP1 model was docked as a dimer, using VP1 models from the A and B positions within the icosahedral lattice. The model was fitted into both the fJAM-1-labeled FCV reconstruction and the unlabeled structure (as a control), using Situs and from a starting orientation defined by the position of this dimer within the crystal structure of SMSV (9). Docking the high-resolution coordinates to the unlabeled structure showed no appreciable movement, whereas there was a clear and consistent rotation of approximately 13° about the local twofold axis of the VP1 dimer in fitting experiments using the labeled structure. Expanding the icosahedral symmetry of the VP1 dimer after docking to the fJAM-1-labeled reconstruction revealed considerable steric collision with the S domains of symmetry-related molecules.

FIG. 1. Stereo pair images of three-dimensional reconstructions of unlabeled (A) and fJAM-1-labeled (B) FCV virions at 16- and 18-Å resolutions, respectively, viewed along the icosahedral twofold symmetry axes. (C) A difference map was calculated by subtracting the unlabeled FCV reconstruction from the fJAM-1-labeled structure; concurrent rendering of the difference map (blue) and unlabeled reconstruction (pink) highlights additional density attached to the P2 domain of the labeled reconstruction. Difference density is also visible in the P1 domain, indicating changes in capsid conformation induced by fJAM-1 binding.

RESULTS

fJAM binds to the outermost face of the FCV VP1 P2 domain. Three-dimensional reconstructions of unlabeled and fJAM-1-labeled FCV virions were calculated at 16 and 18 Å, respectively (Fig. 1A and B). The unlabeled structure exhibits the classical calicivirus morphology, comprising rhomboid, arch-shaped dimeric capsomeres at the icosahedral twofold symmetry axes (C-C dimers) and about the fivefold axes (A-B dimers). The structure is strongly reminiscent of the crystallographically determined structure of SMSV, also a member of the *Vesivirus* genus (9). The C-C dimer appears to be poorly resolved, however, particularly at the region joining the S and P domains, and this may indicate some flexibility in the structure.

The fJAM-1-labeled FCV reconstruction has clearly defined additional density attached to the outer face of the A-B dimers that may be interpreted in the context of the known crystallographic structures of both human and murine JAM-1 (Fig. 1B). The receptor density has twofold rotational symmetry, a fea-

ture consistent with its attachment to a dimer at a local twofold symmetry axis. The additional density at the outer face of the C-C dimers is less readily interpreted. This may reflect the flexibility in the underlying structure indicated by our unlabeled FCV reconstruction.

The fJAM-1 density at the A-B dimer suggests two molecules in a head-to-tail arrangement, with one domain laying flat to the outer surface of the P2 domain of VP1 and the second protruding from the surface (Fig. 1B). At this resolution, the density appears contiguous, which might be interpreted as steric overlap between the two fJAM monomers. It is sometimes the case in icosahedral virus-receptor complexes that bound ligands come into steric collision with and thereby prevent binding of molecules at symmetry-related sites (37, 48). In this case, the density within the fJAM component of the reconstruction is ~80% of the maximum intensity within the capsid, at the A-B dimer. This suggests that receptor occupancy is high and supports the interpretation that steric collision does not prevent two fJAM-1 molecules binding to each capsomere.

Binding of fJAM-1 induces conformational changes in the FCV capsid. Figure 1C shows a difference map, calculated by subtracting the unlabeled FCV reconstruction, filtered to 18 Å, from the fJAM-1-labeled structure. In addition to highlighting the density that we attribute to fJAM-1, this map reveals movements in the P1 region seen as additional density. A second difference map was calculated by subtracting the fJAM-1 component from the fJAM-1-labeled FCV reconstruction, leaving only the capsid (Fig. 2A). Comparison with the unlabeled structure (Fig. 1A and see movie S1 in the supplemental material) and superimposition of the two structures (Fig. 2B) further demonstrates the considerable changes induced within the capsid by fJAM-1 binding. Spherical sections through both labeled and unlabeled reconstructions reveal that these structural rearrangements extend throughout the capsid and are visible in both the P and S domains. Particularly noticeable is a ~13° anticlockwise rotation in the P dimer about its local twofold symmetry axis (Fig. 2C to E and see movies S1 and S2 in the supplemental material). This rotation is not visible in Fig. 2A, owing to the manner in which the difference map is calculated (the subtracted fJAM-1 component also contains density from P2). When viewed along the threefold symmetry axes, features in the S domains in the labeled reconstruction appear to rotate about the symmetry axis by 18° clockwise compared to the unlabeled structure (see movie S1 in the supplemental material). Comparable movement is also seen at the icosahedral fivefold symmetry axes.

Modeling the virus-receptor interaction at high resolution.

There are no published crystallographic data on the capsid of FCV or on fJAM-1. There are, however, crystal structures for the closely related SMSV (PDB 2GH8) (9), which is also a member of the *Vesivirus* genus, and for both human and murine JAM-1. We have therefore constructed homology models for FCV VP1 and fJAM-1 based on these structures (using human JAM-1, PDB 1NBQ) (41) to build a model of the interaction and thereby define regions of virus and receptor that may be important for virus attachment and entry (see Fig. S2 in the supplemental material).

Changes in the relative orientation of VP1 dimers, brought about by fJAM binding, complicate experiments to dock mod-

FIG. 2. (A) The fJAM-1 component of the labeled FCV reconstruction was computationally removed to give a better view of conformational changes to the underlying structure (stereo view A). (B) Superimposition of the unlabeled (pink) and labeled (blue) capsids reveals structural changes in both S and P domains. (C and D) Rotations in the P2 region are not readily visualized in panels A and B. Spherical sections through unlabeled (C) and labeled (D) structures at radii of 155 Å (left) and 200 Å (right) reveal movement in the P2 region and confirm that the structural changes extend from the P2 domain throughout the capsid to the S domain. (E) Overlaid contoured images from these spherical sections further highlight the conformational changes. Contours are colored blue for the labeled structure and pink for the unlabeled capsid. Density appears rotated in both S and P2 (sub)domains.

FIG. 3. (A and B) Docked homology models for FCV VP1 (pink ribbon [A]) and fJAM-1 (blue ribbon [B]) in the 18-Å resolution reconstruction for FCV labeled with fJAM-1 (transparent blue surface). (C and D) Atomic-resolution representation of the modeled structure for a FCV A-B dimer of VP1 (pink spheres) labeled with soluble fJAM-1 (blue and green spheres).

eled VP1 coordinates. Docking the complete capsid asymmetric unit did not yield a reliable model. Close inspection of our reconstructions suggested that the rotation of the P domain might be interpreted as a rigid body motion about the VP1-dimer interface. Several approaches to docking our VP1 model were taken, including fitting individual VP1 monomers, VP1 dimers, VP1 domains, and subdomains, as well as the full asymmetric unit. Visual inspection indicated that the best result was achieved by docking A-B VP1 dimers to the full fJAM-1-labeled FCV reconstruction. The P domain fitted well in the reconstructed density (Fig. 3A) and was rotated approximately 13° relative to the dimer position in the unlabeled structure. In this fit the S domain showed considerable steric collision with symmetry-related VP1 molecules, indicating that VP1 does not rotate as a rigid body. Close inspection of both labeled and unlabeled reconstructions suggests that the conformational changes in the S domain might still result from rigid-body movement of that domain. It seems plausible that the changes in capsid conformation might involve a hinging movement between the P and S domains. Attempts to fit the S domain independently did not lead to models that we judged to be reliable, however, owing to a lack of strong features in the contiguous floor of the capsid. Since the density in the C-C dimer is not well resolved, no fitting was performed to this region.

To position the receptor relative to the docked VP1 A-B dimer, homology-modeled fJAM-1 coordinates were fitted to a difference map calculated by subtracting the unlabeled FCV structure (filtered to 18 Å) from the fJAM-1-labeled structure. The fJAM density bound at the A-B dimer strongly suggested that two molecules bind to each capsomere. This allowed us to

FIG. 4. Dissecting the virus-receptor interface to highlight putative contact residues. (A) Model of an fJAM-1 decorated A-B dimer viewed perpendicular to the capsid surface. (B and C) The receptor component is rotated horizontally 180° to reveal the contact face in both models. Amino acid residues identified as being important for FCV infectivity (34) or conserved in JAM-1 molecules from different species that permit FCV infection in cell culture (29) were found in the contact face of fJAM-1 bound to VP1 at position A (B). Only S91 identified by Makino *et al.* was found in the contact face of fJAM-1 bound to VP1 at position B (C). (D and E) Contact residues within VP1 are mostly located within the N-terminal HVR (43) in both A (D; colored pink and blue) and B (E; colored pink and green) quasiequivalent positions.

perform two fitting experiments, exploiting the local symmetry of the virion and thereby validating our findings. The fJAM density at the C-C dimer was not adequately resolved to interpret through docking experiments. In both instances, fitting of fJAM coordinates to density proximal to VP1 monomers at quasiequivalent positions A and B gave higher correlation values when the D1 domain was bound to the capsid surface, with D2 protruding (Fig. 3B to D), a finding supported by mutagenesis data (34). The two fJAM monomers bound at each A-B

dimer are tilted toward each other, such that D2 is positioned above D1 of the symmetry-related molecule. In our model there is a steric collision between bound ligands in this region, involving a loop at residues 175 to 180. Density measurements suggest that both binding sites are occupied. Therefore, it is likely that some rearrangement in this loop is required to accommodate the two molecules' close proximity to each other. We have previously demonstrated such an event for decay-accelerating factor bound to echovirus type 12 (37).

Description of putative binding surfaces. Our model of the virus-receptor interaction allows us to describe putative contact residues on fJAM-1 and highlights the region of P2 that may be important in virus attachment and entry (Fig. 4). Two fitting experiments were performed with fJAM-1. While these experiments have yielded a model with broadly similar orientations of fJAM-1 relative to the capsid surface, there are some differences. In our docking of high-resolution data to density bound to VP1 in quasi-equivalent position A, the majority of the contact face is located in D1 (Fig. 4B). Interestingly, residues identified as being important for FCV infectivity (34) are present within the contact face. Makino et al. highlighted two residues that are conserved between feline and simian JAM-1 but not in human JAM-1 (feline and simian JAM-1 both permit infection by FCV), S91, and K155. The former is located within the contact face (29). Our second fit, to fJAM-1 bound to VP1 in quasi-equivalent position B, shows greater contact with D2. In this fit the ligand leans slightly more toward the symmetry-related fJAM-1 molecule. Putative contact residues identified by Ossiboff et al. (34) are not within the contact face, while S91 remains present (Fig. 4C).

Contact residues within VP1 cover a significant portion of the outer face of the P2 domain (Fig. 4D and E). This region has been shown to exhibit considerable variation between serotypes and is also the region containing the major antigenic determinants of FCV. Two hypervariable regions (HVRs) have been identified within P2, an N-terminal HVR at residues 426 to 460 and a C-terminal HVR at 490 to 520 (43). A conserved region flanked by these two HVRs is suggested to be involved in dimer formation (9). In both docking experiments the majority of the contact face resides within the N-terminal HVR. Despite the apparent differences in fJAM-1 orientation between these two fits, S91, identified as possibly important for FCV binding (29), contacts a conserved tyrosine at position 435 in both instances.

DISCUSSION

We have determined the structure of FCV strain F9 bound to its cellular receptor fJAM-1 at 18-Å resolution by cryo-EM and three-dimensional image reconstruction. Our study provides the first structural view of a native calicivirus-protein receptor complex, revealing conformational changes to the capsid induced by receptor binding. We have used computational methods to derive a high-resolution model of the interaction, demonstrating that the fJAM-1 D1 domain binds to the outer face of the P2 domain of the viral capsid protein VP1. Furthermore, we have identified putative contact surfaces for both virus and receptor that incorporate specific amino acids or regions that have been shown to be important in virus attachment or neutralization.

Conformational changes in the FCV capsid: a prelude to uncoating? Our reconstructions demonstrate significant conformational changes induced by fJAM-1 binding to the FCV capsid, leading us to suggest that these may indicate a process of receptor-mediated uncoating. In vitro experiments such as those described here probably involve suprastoichiometric quantities of receptor, such that the entire capsid is decorated, inducing gross conformational changes not found in vivo. At

the cell surface fJAM-1 might bind to a smaller number of capsomeres, inducing local conformational changes necessary to release the genome into the cell, perhaps through the formation of a pore, as has been suggested for poliovirus (7). Indeed, studies have indicated that FCV infection permeabilizes cell membranes, allowing the entry of toxins such as α -sarcin, supporting the suggestion of pore formation (45). Purification of virions from infected CRFK cells has identified two density populations: heavy particles (1.33 g/ml) and light particles (1.22 g/ml) (51). The latter particles are not infectious and do not contain viral genomes and, as such, may reflect a population of "postentry" capsids as proposed for similar particles isolated from picornavirus-infected cells (22). FCV has been shown to enter cells through clathrin-mediated endocytosis, and experiments have indicated that endosome acidification is important (45). It is possible then that the FCV-fJAM-1 interactions might prime the virus for the low pH environment of the endosome, allowing efficient uncoating to occur. Such receptor priming has previously been reported for enveloped viruses such as avian leukosis virus (32), as well as nonenveloped viruses such as the major rhinoviruses (33).

At the resolutions obtained here, we are unable to provide structural information describing conformational changes at the tertiary structure level. To completely and reliably fit VP1 to cryo-EM data will require a flexible approach incorporating molecular dynamics and secondary structure detection. This will require considerably higher resolution (4 to 6 Å), and indeed this will also be necessary to confirm the apparently slightly different binding orientations for fJAM bound to VP1 at quasi-equivalent positions A and B.

Viruses interact with JAM-1 in a monomeric state. Crystallographic studies of human and murine forms of JAM-1 have indicated that these molecules form dimers, and it has been suggested that the dimeric form may be functionally important. Our data do not suggest the existence of a similar dimeric form of fJAM-1 in solution. Mutagenesis studies of reovirus attachment to hJAM-1 indicate that the interaction involves a monomeric form of the receptor. Amino acid residues implicated in reovirus binding by these investigations are found within the dimer interface of hJAM-1 (17). They are not located within the contact face we have identified for FCV binding however, although they are within D1. The putative dimer interface and FCV binding site do not overlap, suggesting that the virus-receptor interaction does not compete or interfere with a native dimeric state in fJAM-1.

Viruses interactions with Ig-like cell adhesion molecules and cell surface glycans. Many viruses bind to Ig-like cell adhesion molecules (5, 6, 13, 21, 38, 47). The localization of these proteins to tight junctions might be expected to present a barrier to virus infection. Group B coxsackieviruses use CAR as a cellular receptor. Like JAM-1, CAR is located in the tight-junction of epithelial cells and is presumed to be inaccessible. Group B coxsackieviruses have been shown to access CAR through an intermediary interaction with the complement control protein decay-accelerating factor. This interaction activates cellular pathways, leading to actin rearrangement and trafficking of virus to the tight-junction, permitting virus entry (11).

Makino et al.'s description of the interaction between FCV and fJAM-1 is the first study revealing a protein receptor for a

member of the *Caliciviridae*. Many investigators have highlighted the importance of interactions with cell surface glycans such as sialic acid in this virus family (19, 31). Indeed, FCV also binds to α 2,6-sialic acid (44). Considering the above-mentioned processes found in coxsackieviruses, an interesting parallel might be drawn between FCV interactions with JAM-1 and sialic acid and those of reoviruses. Reoviruses exhibit serotype-dependent tropism, while all strains are capable of infecting cells expressing JAM-1. It has been suggested that tissue-specific cell surface carbohydrate may influence tropism in these viruses (5). Moreover, a two-step attachment process has been proposed for reovirus involving initial attachment to cell surface sialic acid, followed by interaction with JAM-1 (4). It is possible then that cell surface glycans, such as α 2,6-sialic acid in the case of FCV and histo-blood group antigens in the case of the noroviruses, are the sites of initial attachment and principal determinants of viral tropism. Subsequent diffusion or trafficking of bound virus across the cell surface may then lead to virus entry after interactions with specific protein receptors such as JAM-1 located in tight junctions. Alternatively, sialic acid within the carbohydrate moieties on fJAM-1 or other tight-junction proteins may play a more straightforward role in stabilizing the interaction between fJAM-1 and FCV. However, the observation that fJAM-1 expressed in bacterial cells, which should lack any form of glycosylation, inhibits virus binding indicates that, at least for certain strains, the carbohydrate moiety of fJAM-1 is not required for receptor binding (34).

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