Antibody-induced uncoating of human rhinovirus B14

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Rhinoviruses (RVs) are the major causes of common colds in humans. They have a nonenveloped, icosahedral capsid surrounding a positive-strand RNA genome. Here we report that the antigen-binding (Fab) fragment of a neutralizing antibody (C5) can trigger genome release from RV-B14 to form emptied particles and neutralize virus infection. Using cryo-electron microscopy, structures of the C5 Fab complex in the full and emptied particles have been determined at 2.3 Å and 3.0 Å resolution, respectively. Each of the 60 Fab molecules binds primarily to a region on viral protein 3 (VP3). Binding of the C5 Fabs to RV-B14 results in significant conformational changes around holes in the capsid through which the viral RNA might exit. These results are so far the highest resolution view of an antibody–virus complex and elucidate a mechanism whereby antibodies neutralize RVs and related viruses by inducing virus uncoating.

Rhinoviruses (RVs) belong to the Enterovirus (EV) genus of the Picornaviridae. The family consists of a diversity of nonenveloped, icosahedral viruses that possess a single strand of positive-sense RNA genome (1). Specifically, these viruses include a number of medically important human pathogens, such as RVs, foot-and-mouth disease virus, polioviruses, coxsackieviruses, echoviruses, EV-D68, EV-A71, and hepatitis A virus (2). Infection of RVs leads to both acute upper and lower respiratory tract diseases in humans, which cause a heavy burden for public health finance (3). There are three species of RVs based on sequence comparisons (RV-A, -B, and -C). Members of the species RV-A and RV-B have also been classified into two groups (major and minor) based on their receptor use for cell entry (4). The major group of RVs (e.g., RV-B14) use intercellular adhesion molecule 1 (ICAM-1), an Ig-like molecule, as a receptor, and the minor group RVs (e.g., RV-A2) use low-density lipoprotein receptor as a receptor. In contrast, members of the recently identified RV-C species use cadherin-related family member 3 as a cellular receptor (5).

The genomic RNA of RVs and other related picornaviruses encodes four capsid proteins, VP1–VP4. The capsid of these viruses has a size of ~300 Å in diameter and exhibits pseudo-T = 3 icosahedral symmetry (6). The icosahedral capsid shell is formed by 60 copies of VP1, VP2, and VP3, of which the N termini are located in the virus interior and the C termini decorate the virus external surface. These three polypeptides possess an eight-stranded anti-parallel β-barrel “jelly roll” fold (7). In contrast, VP4 has an extended structure, and 60 copies of VP4 reside inside the capsid. In addition, the capsid shows a star-shaped mesa on each fivefold vertex that is surrounded by a ~25 Å deep canyon. The capsoid region of major receptor group RVs is responsible for binding ICAM-1 (8).

Binding of ICAM-1 to RV-B14, as also binding of other Ig-like receptors to their respective EVs, at physiological temperatures results in dramatic structural rearrangements of the capsid that triggers virus uncoating (9, 10). Upon attachment onto host cells via receptor binding, the native infectious virions (150S) are converted into A(altered)-particles (135S), which are characterized by the loss of VP4 and externalization of the VP1 N-terminal residues (11). The A-particles were proposed to release the genomic RNA within host cells and to become emptied B-particles (80S) (12, 13). Thus, the A-particles were considered to be an intermediate state in the uncoating pathway (14). Therefore, conformational changes of the capsid are crucial to viral entry and are triggered by interaction with the host cell.

Host humoral immune response, based on antibodies, represents a major defense line against virus infections. Antibodies can neutralize virus infections by binding to specific regions, namely epitopes, on the virus outer surface. Nonenveloped viruses, including picornaviruses, can be neutralized by antibodies that prevent virus attachment onto host cells and/or stabilize the virus to impede virus uncoating (15). A recent study reported that a neutralizing antibody E18 caused genome release of EV-A71, thereby discovering a new mechanism of neutralization (16). Nevertheless, the current understanding of antibody-induced uncoating is largely limited by a lack of high-resolution structures of picornavirus–antibody complexes. Furthermore, it remains unknown whether antibody-induced virus uncoating is a mechanism shared by many picornaviruses.

In the present work, cryo-electron microscopy (cryo-EM) analyses were performed using RV-B14 and a marine neutralizing monoclonal antibody (mAb) C5, which had previously been proposed to bind the neutralizing immunogenic (Nl)-III site on the RV-B14 outer surface (6). The Nl-II site is located primarily on VP3 and represents one of the four major Nl sites on RVs, as determined by studies of neutralizing antibody escape mutants using a panel of mAbs against RV-B14 (17, 18).

Significance

Rhinoviruses (RVs) frequently cause respiratory infections in humans. However, there are currently no approved antiviral treatments or vaccines available. Using cryo-electron microscopy (cryo-EM), we have shown that the antigen-binding fragment of a neutralizing antibody, C5, binds to RV-B14 and causes significant structural rearrangements of the capsid to allow release of the viral genome. C5-induced virus uncoating depends upon temperature and virus–Fab molar ratio. These results present so far the highest resolution cryo-EM structures of a virus–antibody complex and suggest a conserved mechanism whereby antibodies neutralize infections by RVs and related viruses via induction of premature genome release from the capsid.

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Data deposition: The coordinates of set-a-full, set-a-empty, set-b-full, and set-c-full have been deposited to the Protein Data Bank, www.pdb.org (PDB ID codes 5W3E, 5W3O, 5W3L, and 5W3M, respectively). The cryo-EM maps for set-a-full, set-a-empty, set-b-full, and set-c-full have been deposited to the Electron Microscopy Data Bank (entry nos. EMD-8754, EMD-8763, EMD-8761, and EMD-8762, respectively).

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Here we show that binding of the antigen-binding (Fab) fragments of C5 to RV-B14 causes native full virions to release the genomic RNA in a temperature- and virus–Fab molecular ratio-dependent manner. During Fab-induced uncoating, the capsid undergoes particle expansion to form pores at twofold axes through which the viral RNA might exit. Our results suggest that C5 Fab might preferentially select the expanded conformation of the capsid over the unexpanded conformation to facilitate virus uncoating.

Results and Discussion

Cryo-EM Reconstructions of RV-B14 Complexed with C5 Fab. The Fab fragment of C5 was incubated with RV-B14 under three different conditions by varying the incubation temperature and virus–Fab molar ratio. This resulted in three cryo-EM datasets (set-a, set-b, and set-c) (Table S1). When the Fab and the virus were incubated with a ratio of ∼180 Fab per virion at 33 °C for 1 h (dataset set-a), EM micrographs showed the presence of a mixed population of full and empty particles. There were 30–60% of empty particles as estimated by visual inspection of the micrographs (Fig. S1). Furthermore, 2D classification of individual particle images extracted from the micrographs separated full (set-a-full) and empty (set-a-empty) particles and showed prominent spikes on the outer surface of both types of particles (Fig. S1). This indicated that both types of particles were bound by Fab molecules. In contrast, when the temperature was changed to 4 °C (set-b) or the molar ratio was changed to ∼60 Fab per virion (set-c), empty particles that showed spike-like densities on the particle outer surface only accounted for less than 0.5% of all particles.

The observation that full and empty particles coexist under the same incubation condition (set-a) provides a basis for comparison. If the molar ratio (Fabs per binding site) is decreased from 3:1 (set-a) to 1:1 (set-b), the Fab molecules are unable to cause the virus to lose its genome. Similarly, if the temperature is decreased while keeping the molar ratio the same (set-a to set-c), then also the Fab fails to cause release of the genome. These results demonstrate that C5 Fab can induce genome release of RV-B14 in a temperature- and virus–Fab molar ratio-dependent manner.

After 2D classification, a dataset of 46,684 full particles (set-a-full) and a dataset of 9,521 empty particles (set-a-empty) were selected from set-a; also, 23,090 full particles (set-b-full) were selected from set-b, and 36,866 particles (set-c-full) were selected from set-c. A “gold standard” single particle icosahedral reconstruction strategy was independently applied to each of these four sets of particle images using the program jspr (19, 20). High-resolution refinements involved parameters that define particle orientation, particle center, phase error due to beam tilt, astigmatism, defocus, magnification, and anisotropic magnification distortion. The resolutions of the final reconstructions are 2.5 Å for set-a-full, 3.0 Å for set-a-empty, 2.7 Å for set-b-full, and 2.3 Å for set-c-full (Fig. 1 and Figs. S1–S3), respectively, as determined by the Fourier shell correlation (FSC) between two half maps using the criterion of FSC = 0.143 (21).

Conformational Changes Associated with Viral Genome Release. The capsid structures of all three full particle–Fab complexes are essentially the same, as is evident from the root-mean-square deviation (rmsd = 0.2–0.7 Å) between equivalent Ca atoms for each pair of structures when icosahedral symmetry axes are aligned (Table S2). In contrast, the capsid structure of the empty particle–Fab complex is significantly different from any of the full-particle structures (rmsd = 5.5–6.1 Å) (Table S2). The VP1 N-terminal residues 1001–1060, VP2 residues 2043–2056, VP3 residues 3172–3181, and the VP4 residues are disordered in empty particles. (Residues are numbered by adding 1,000 to VP1 residues, 2,000 to VP2 residues, 3,000 to VP3 residues, and 4,000 to VP4 residues.) These observations suggest that the VP1 N-terminal residues might be externalized and that VP4 might be lost from the empty particle, because these residues reside in the interior of the full particle. It has been suggested that externalization of these internal residues facilitates interactions of the capsid with host cell membranes during cell entry of EVs (22, 23).

The empty particle structure (set-a-empty) is expanded by about 12 Å in diameter with respect to the full-particle structure (set-a-full). This expansion is caused primarily by movements of VP1, VP2, and VP3 as individual rigid bodies, because the jelly roll β-barrel of each capsid protein in the full-particle structure can be superimposed with its counterpart in the empty particle structure with rmsds of 1.0 Å (VP1), 0.5 Å (VP2), and 0.9 Å (VP3) (Fig. S4). Specifically, VP1, VP2, and VP3 are moved away from the virus center by 6.6 Å, 3.9 Å, and 5.1 Å and rotated by 2.5°, 5.7°, and 4.3°, respectively (Table 1). As a consequence, an α-helix in VP2 (residues 2091–2098) and its twofold-related helix move away from each other. These structural changes lead to the formation of roughly rectangular (25 Å × 8 Å) pores around the icosahedral twofold axes through which the viral RNA and VP4 might exit (Figs. 1 and 2) (24, 25). In addition, because of the rigid body movements and the disordering of residues 3172–3181 in the VP3 GH loop (the loop that links β-strands G and H), pores are opened up near the quasi-threefold axes (Fig. 1), which probably allow for the externalization of VP1 N-terminal residues as previously suggested (26, 27).

The buried surface areas at the interface between any pair of capsid proteins within one protomer (VP1, VP2, and VP3 as defined in ref. 6) of the empty particle are similar to those in the full particle (Table S3). Thus, the respective protomers in these two forms of particles were superimposed for structural comparison. Within the protomer, VP2, VP3, and the fivefold distant regions of VP1 in the two structures are well aligned to each other, with an rmsd of 1.4 Å. Nevertheless, the jelly roll β-barrel and fivefold proximal loops of VP1 undergo a hinge-like motion with a translation of 1.1 Å and a rotation of 7.5°, which helps maintain interactions between amino acid residues near the fivefold axes. Furthermore, the VP2 C-terminal tail (residues 2253–2260) near each twofold axis is located on the outer surface of the full particle. In the empty particle, the tail is displaced by an rmsd of 17.7 Å between equivalent Ca atoms (Fig. S5). These residues are internalized and interact with VP3 in the neighboring, fivefold-related protomer and with VP2 in the neighboring, twofold-related protomer (Fig. 2). As a result, the...
 tail participates in forming the aforementioned pores at twofold axes and probably contributes to stabilize the particle. Such structural change of the VP2 C-terminal tail has not been previously observed in other A-particle or empty particle-like structures of EVs (13, 27).

In all three full-particle maps, but not in the empty-particle map, there is a region together with its icosahedral twofold-related counterpart that forms density around each twofold axis at the capsid inner surface. The density height of this region is about 4 σ above the mean of the cryo-EM map (set-c-full, unsharpened map), whereas densities for the main chain of surrounding amino acids are about 13 σ high. This region, which probably represents ordered viral RNA, can accommodate about six ribonucleotide residues. In addition, it is in close proximity to the VP1 N-terminal residues 1022–1027 and the VP2 N-terminal residues 2037–2039 (Fig. 2). These observations reinforce the suggestion that the viral RNA probably exists through the pore at a twofold axis upon externalization of internal residues including the VP1 N-terminal residues.

**Interactions between RV-B14 and C5 Fab.** The 2.3 Å resolution cryo-EM map of set-c-full shows well-ordered densities of residue side chains at the binding interface between C5 Fab and the capsid (Fig. S6). Despite a lack of experimentally determined sequence information on C5, a close-to-authentic amino acid sequence of the variable region was generated by taking advantage of high-resolution map density features and 499 homologous X-ray crystal structures of murine antibodies (Materials and Methods).

In all four structures of RV-B14 complexed with C5 Fab, nearly the same set of capsid protein residues is identified to interact with the variable heavy (VH) domain and variable light (VL) domain of C5. Each of the 60 Fab molecules binds to only one protomer by contacting a threefold proximal region on the virus outer surface (Fig. 3A). This region, in which the NIm-III site is covered, is formed mainly by residues in the VP1 C terminus, VP2 C terminus, VP3 N terminus, VP3 BC loop, and VP3 H1 loop (Fig. S7). The footprint of C5 Fab on RV-B14 is similar to that of E18 Fab on EV-A71 (16). Both Fabs act as an inducer of virus uncoating in vitro and neutralize virus infection in cell-based plaque reduction neutralization tests (Fig. 3B). These observations suggest that NIm-III—binding neutralizing antibodies might use a common neutralizing mechanism by triggering genome release of EVs. In contrast, previous structural studies on antibodies that target other NIm sites on RV-B14 or homologous sites on other EVs have not shown that these antibodies cause virus uncoating (28–32).

**Mechanism of C5 Fab-Induced Virus Uncoating.** C5 Fab has a footprint on RV-B14 that does not have any overlap with that of ICAM-1 (Fig. S8). Nor do any of the 60 Fab molecules clash with ICAM-1 when an ICAM-1 molecule (Ig-like domains 1–2) is docked onto the C5 Fab-bound full particle (Fig. S8). Nevertheless, the temperature dependency of C5 Fab-triggered genome release is reminiscent of ICAM-1–induced uncoating of RV-B14 and related RVs in vitro (10, 33). In both cases, virus uncoating does not or rarely occurs at 4 °C, whereas the capsid becomes expanded upon receptor or Fab binding at physiological temperatures. This is due in large part to the observation that physiological temperatures favor virus breathing. In this process, the virus reversibly externalizes internal amino acid residues and undergoes particle expansion/shrinkage (34). Furthermore, as in the crystal structure of RV-B14 (6), the full-particle structures presented here do not contain a “pocket factor” in the hydrophobic pocket of the VP1 jelly roll. Because the pocket factor, a fatty acid-like molecule, contributes to stabilizing many other EVs (35–37), the absence of this stabilizing factor in RV-B14 would facilitate virus breathing at physiological temperatures. Thus, it is probable that binding of C5 Fab to the virus, much like receptor binding to the virus, shifts the equilibrium to the expanded conformation, which might have a higher affinity to C5 Fabs than the nonexpanded conformation (38). In this way, C5 Fab can lead to irreversible structural changes of the virus and induce virus uncoating, as does receptor binding (38).

Unlike ICAM-1, binding of C5 Fab to the virus at physiological temperatures does not lead to the formation of an A-particle or A-particle–like intermediate state, which exhibits an expanded capsid that contains the viral RNA (11, 27). This observation indicates that a higher activation energy barrier probably exists in the process of C5-Fab–induced virus uncoating than that required for ICAM-1–induced virus uncoating. Compatible with this prediction, a threshold of virus–C5 Fab molar ratio between 1:60 and

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**Table 1. Translation and rotation of individual capsid proteins during virus uncoating**

| Protein | Rmsd, Å | Translation, Å | Rotation,° | ψ,° | ϕ,° |
|---------|---------|----------------|------------|------|------|
| VP1     | 1.0     | 6.6            | 2.5        | 74.8 | 30.8 |
| VP2     | 0.5     | 3.9            | 5.7        | 61.6 | 12.0 |
| VP3     | 0.9     | 5.1            | 4.3        | 76.4 | 12.0 |

*aBased on equivalent Cα atoms in the jelly roll β-barrel between the full and empty particles.

*bCounter-clockwise rotation.

*cThe direction of the rotational axis is defined by these two polar angles according to ref. 63.

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**Fig. 2.** Pores around the icosahedral twofold axes in the empty particle. Comparison of the twofold proximal regions of the full (A) and empty (B) particles. The ribbon diagrams are colored: VP2 C-terminal residues 2253–2260 (blue) and the remaining residues of VP2 (green) and VP3 (red). (C) Density at the capsid’s inner surface in the full particles around the twofold axes probably represents ordered RNA. A yellow contour outlines the density when looking out from the inside of the particle along a twofold axis. Surrounding residues are colored blue (VP1) and green (VP2).
Materials and Methods

Virus Growth and Purification. RV-814 was grown in HeLa-H1 (ATCC CRL195) cells and purified as described previously (42, 43). Briefly, cells were infected by RV-814 (multiplicity of infection = 2) for 24–36 h until complete cytopathic effect was observed. The supernatant and infected cells were separated by centrifugation. The cell pellets were subjected to four freeze–thaw cycles followed by 30 s of Bounce homogenization. Upon centrifugation and removal of cell debris, the virus-containing supernatant was combined with the aforementioned supernatant and spun down at 277,937 × g for 2 h using a Beckman Ti 50.2 rotor. The resultant pellets were resuspended in buffer 1:180 is necessary to cross the energy barrier and to cause virus uncoating (Table S1). In contrast, a single Ig-like receptor molecule can induce local structural rearrangements of an EV near the receptor binding site that primes the virus for uncoating (39). An analogous process would be anticipated when ICAM-1 interacts with RV-B14. Nevertheless, it is currently not clear what accounts for these differences between C5 Fab and ICAM-1–triggered virus uncoating, which awaits future investigations.

In summary, the present work has extended the discovery of antibody-caused viral genome release to another member of the EV, RV-B14. Thus, antibody-induced uncoating might be a conserved mechanism for many EVs. The high-resolution cryo-EM structures presented here provide the molecular basis for the design of immunogens to elicit antibodies that neutralize EV infections via induction of premature virus uncoating.
a physical pixel size of 1.30 Å per pixel at the specimen level and with a
defocus range of 0.7–5.8 μm. The dose rate was ~8 e⁻/pixels. A total dose of
~33 e⁻/Å² was fractionated into 38 frames with a frame rate of 200 ms. Sta-
tistics for data collection were summarized in Table S1.

**Image Processing.** The following procedures were performed using programs
as integrated in the image processing pipeline Appion (46). To correct the
stage drift and beam-induced motion during exposure, individual frames of
each movie were aligned using the program MotionCorr (47). All of the
aligned frames were summed up to produce individual micrographs.
The micrographs that had severe drift and ice contamination were discarded
based upon inspection of the power spectra. Contrast transfer function (CTF)
parameters were then estimated using the program CTFFINN3 (48). Particles
were automatically picked up on each micrograph using templates that
were generated based on a small set of manually selected particles. A combina-
tion of the programs FindEM (49) and DoG picker (50) was used in this
process. Upon particle boxing and extraction, a total of 60,065, 26,759,
and 39,728 particles were obtained for set-a, set-b, and set-c, respectively.
For each of the three sets, the particle images were eight times (set-c) or
four times (set-a and set-b) binned and subsequently subjected to reference-
free 2D classification using the program Relion (51), which sorted out junk
particles and separated empty particles from full particles. This produced
four datasets: set-a-full (46,684 full particles from set-a), set-a-empty
(9,521 empty particles from set-a), set-b-full (23,090 full particles from set-b),
and set-c-full (36,866 full particles from set-c). A gold-standard 3D recon-
struction strategy was applied to each of these four datasets using the program
jpr3 as previously described (19, 52). Icosahedral symmetry was imposed in
this process. Every dataset was divided into two halves. The following
procedures were the same for the two halves. An initial model was generated
from experimental images. The parameters for particle orientation and
center of each experimental particle image were refined against the
initial model. A new 3D model was reconstructed from particle images
using the newly determined particle orientation and center. Iterative re-
finement cycles improved the accuracy of particle orientation and center
determination. The refinement procedures were extended to four times
binned, two times binned, and unbinned data. Finally, parameters for par-
ticle orientation, particle center, astigmatism, defocus, magnification, phase
error due to beam shift, and anisotropic magnification distortion (53) were
included in refinement. To achieve 3D reconstructions with the highest
possible resolution, particle images were re-extracted from micrographs that
were generated by summing up the aligned frames 4–49 (set-a and set-b) or
frames 3–16 (set-c). The final map was obtained by averaging the two in-
dependent half maps in real space and sharpened with a negative B factor and
with an FSC curve-based low-pass filter using the program Relion (21,
51). The map resolution was estimated based on the FSC (0.143 as a cutoff)
between the two half maps (unsharpened) (21). The statistics are summa-
rized in Table S1.

**Atomic Model Building and Refinement.** The crystal structure of RV-B14
[Protein Data Bank (PDB) ID code 4RHV] was manually fitted into the final
cryo-EM analysis; and Sheryl Kelly for help preparing this manuscript. Y.D. was
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