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Structural and Molecular Basis for Ebola Virus Neutralization by Protective Human Antibodies

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Abstract

Ebola virus causes hemorrhagic fever with a high mortality rate and for which there is no approved therapy. Two human monoclonal antibodies, mAb100 and mAb114, in combination protect non-human primates against all signs of Ebola virus disease, including viremia. Here, we demonstrate that mAb100 recognizes the base of the Ebola virus glycoprotein (GP) trimer, occludes access to the cathepsin-cleavage loop, and prevents the proteolytic cleavage of GP that is required for virus entry. We show that mAb114 interacts with the glycan cap and inner chalice of GP, remains associated following proteolytic removal of the glycan cap, and inhibits binding of cleaved GP to

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Supplementary Materials

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Materials and Methods

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Table S1

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its receptor. These results define the basis of neutralization for two protective antibodies and may facilitate development of therapies and vaccines.

Main Text

Ebola virus (EBOV) causes a rapidly fatal hemorrhagic fever for which there is currently no treatment (1–3). We recently isolated two antibodies (mAb100 and mAb114) from a 1995 Kikwit Ebola survivor that potently neutralize multiple EBOV isolates spanning over 40 years (4). When administered as a cocktail to rhesus macaques, these antibodies fully protected from clinical symptoms, viremia and death. Furthermore, mAb114 monotherapy fully protected macaques from death and illness when given as late as five days after infection (4). In this study, we sought to identify the structural and molecular basis of neutralization for these protective antibodies.

The EBOV glycoprotein (GP) is a class I fusion protein comprising disulfide-linked subunits, GP1 and GP2, which associate to form a chalice-shaped trimer (5–7). The GP1 subunit binds to the EBOV receptor, Niemann-Pick C1 (NPC1), allowing GP2-mediated fusion of the viral and host-cell membranes (5, 8–11). The GP1 subunit contains a core domain and a “glycan cap”, which are shielded by the heavily glycosylated mucin-like domain (MLD) (Fig. 1A). The MLD is dispensable for virus entry, but is a target for host antibody responses (6, 7, 12–16). Using immunoprecipitation (IP), we found that mAb100 and mAb114 recognized GP ectodomains lacking the MLD (GP_{Muc}), suggesting that their epitopes reside elsewhere on GP (Fig. 1B) (17).

To identify the epitopes recognized by these antibodies, crystal structures of their antigen-binding fragments (Fab100 and Fab114) were determined individually to 2.0 Å and in a ternary complex with GP_{Muc} to 6.7 Å (Table S1, Fig. S1A and B). The complex structure was solved by molecular replacement using the refined structures of the unbound Fabs and the previously solved EBOV GP_{Muc} structure (6) as search models and was refined to an $R_{\text{work}}/R_{\text{free}}$ of 26.0%/34.3% (Table S1). The crystal structure shows that Fab100 binds to the base of GP, parallel to the viral membrane, makes contacts with both GP1 and GP2, and crosslinks two adjacent protomers (Fig. 1C, Fig. S1C). In contrast, Fab114 binds within the GP chalice, perpendicular to the viral membrane, and makes contacts with both the glycan cap and the GP1 core (Fig. 1C, Fig. S1C).

Since GP binds NPC1 in acidic late-endosomes and lysosomes (8–12, 16), we compared antibody binding to GP_{Muc} at neutral and low pH using cryo-electron microscopy (cryo-EM). The structures of the ternary complexes at pH 7.4 and 5.0 were calculated to a resolution of 9 Å by single-particle reconstruction (Fig. S2). The ternary-complex crystal structure fit well as a rigid body into the cryo-EM densities (Fig. 2). Further rigid-body refinement of the Fabs and GP did not change the overall structure substantially, indicating that the crystal structure closely resembles the cryo-EM structure. Comparisons of the cryo-EM structures at pH 7.4 and 5.0 revealed highly similar structures (Fig. 2, Fig. S2C), suggesting that these antibodies would remain associated with GP during trafficking of EBOV to low-pH compartments. Analysis of the cryo-EM structure also revealed a bulky density near the Fab100 interface that would be consistent with an *N*-linked glycan at

residue Asn563 of GP (6, 7) (Fig. S3A). Enzymatic trimming of the glycans using endoglycosidase H (EndoH) did not appreciably alter Fab100 binding to GP, suggesting that *N*-linked glycans are not critical for Fab100 recognition (Fig. S4 A–C).

Binding of Fab100 to the base of the GP trimer (Fig. 1C, Fig. S1C) resembles that of KZ52, a prototypic neutralizing antibody that does not confer protection in macaques (6, 18, 19). However, Fab100 is rotated about the trimeric axis by approximately 60° with respect to KZ52 (Fig. S3B). This rotation enables Fab100 to contact GP1 and GP2 of one protomer, as well as the internal fusion loop (IFL) of the neighboring protomer, whereas KZ52 contacts only a single protomer (Fig. S3C). Fab100 is also in close proximity to the β 13– β 14 loop (Fig. 3A) (residues 190–213), which is disordered in the previous structure (6). Biochemical studies have shown that EBOV entry requires cleavage of this loop by cathepsin (Cat) L and B (11, 12, 20–23), which releases the glycan cap and MLD, exposing the receptor-binding domain (RBD) within the GP1 core (8–12, 20, 21). Interestingly, the cryo-EM structure revealed additional electron density in close proximity to the Fab100 light chain corresponding to portions of the β 13– β 14 loop (Fig. 3A). The observed density would be expected to accommodate residues 190–197 and 209–213, and may accommodate additional residues depending on the conformation of the β 13– β 14 loop, which is difficult to determine given the weak electron density of this region. We therefore hypothesized that Fab100 would sterically block proteolysis by cathepsins. To test this, we used Cat L to digest GP_{Muc} that was pretreated with mAb114, mAb100, KZ52 or control mAb. The amount of cleaved GP (GP_{20k}) was similar in the control and mAb114 reactions (Fig. 3B). Consistent with previous reports, KZ52 delayed the appearance of GP_{20k} (24). For mAb100, the primary product was an intermediate form (GP_i) with only trace amounts of GP_{20k}, indicating that mAb100 significantly reduced GP cleavage (Fig. 3B). Similarly, cleavage of GP by thermolysin, which mimics Cat B (8, 11, 20, 23), was inhibited by mAb100 (Fig. S4D). These data suggest that mAb100 neutralizes EBOV by sterically blocking cathepsin cleavage of the β 13– β 14 loop.

We next determined the affinity of Fab100 and KZ52 for GP_{Muc} at neutral and low pH. The affinity of Fab100 was ~5- and ~10-fold stronger than KZ52 at pH 7.4 and 5.3, respectively (Fig. 3C, Fig. S4A), suggesting that mAb100 remains tightly associated with GP in low pH compartments. Notably, the affinity of KZ52 for thermolysin cleaved GP (GP_{THL}) at pH 5.3 was decreased by over 1,000-fold as compared to uncleaved GP. For Fab100, a modestly reduced affinity for GP_{THL} was driven primarily by an increased k_{off} (Fig. 3C, Fig. S4A). However, the k_{off} of the bivalent mAb100 IgG was similar between uncleaved and GP_{THL} (Fig. S5), suggesting that mAb100 remains bound even after proteolytic cleavage at low pH. Due to the quaternary nature of the mAb100 epitope, which includes the internal fusion loop, mAb100 may also prevent conformational rearrangements of GP that occur downstream of proteolytic cleavage. Antibodies with quaternary epitopes have been recently identified that potently neutralize other viruses, suggesting that this mode of binding represents a powerful immunological solution to viral entry (25–27).

Unlike mAb100, mAb114 recognizes an epitope spanning both the glycan cap and the GP1 core (Fig. 4A, Fig. 1C). Most contacts appear to be made between the CDR H3 and L3 of Fab114 and the loop connecting β 8 and β 9 of the GP1 core (6). The importance of this

region for mAb114 binding was confirmed by CLIPS conformational-epitope mapping (28) (Fig. S6A). Since the contacts within the GP1 core remain following cathepsin cleavage (Fig. S7A), we investigated mAb114 binding to GP_{THL} using IP and found that GP_{THL} was recognized similarly to GP and GP_{Muc} (Fig. 4B). Furthermore, negative-stain EM showed that the binding orientation of Fab114 to GP_{THL} was similar to GP_{Muc} (Fig. 4C), demonstrating that the glycan cap is dispensable for mAb114–GP interaction.

Since EBOV particles transit from neutral to low-pH compartments (12, 16, 20), we measured binding kinetics of Fab114 to GP_{Muc} at pH 7.4 and 5.3 and observed similar sub-nanomolar affinities (Fig. 4D, Fig S6B). When compared to antibody 13C6, which competes with mAb114 for binding to GP (4) but is not protective in macaques (15, 29), Fab114 has a significantly slower k_{off} , leading to an affinity that is over 250- and 40-fold tighter at pH 7.4 and 5.3, respectively (Fig. 4D, Fig S6B). Importantly, 13C6 only makes contact with the glycan cap (30), whereas mAb114 bound GP following glycan cap removal (Fig 4B–C). The affinity of Fab114 for GP_{THL} at pH 5.3 remained high (K_D of 8.0 nM), consistent with our structural data showing that Fab114 primarily contacts residues within the GP1 core.

Following cathepsin cleavage of GP1, a hydrophobic pocket on the GP1 core that is formed primarily by $\alpha 1$ and $\beta 4$ and bordered by charged residues in $\beta 7$, $\beta 8$, and $\beta 9$ is exposed. These regions have been proposed to be the RBD of EBOV GP (6, 23, 31–36). Our data suggested that the interaction of mAb114 with residues in $\beta 7$ – $\beta 9$ might block NPC1 access to this pocket. To test this, we performed a competition assay with mAb114, GP_{THL}, and NPC1 domain C (NPC1-dC)—the domain responsible for engaging cleaved GP and mediating virus entry (8–10). Using biolayer interferometry, we found that when mAb114 was bound to GP_{THL}, NPC1-dC was unable to bind (Fig. 4E, Fig S6C). Similar results were obtained using IP (Figure S6D). These findings are consistent with the observation that both Fab114 and NPC1-dC have similar affinities for GP_{THL} (Fig. 4D, Fig S6B) and indicate that mAb114 neutralizes EBOV infection by preventing binding of cathepsin-cleaved GP to its receptor NPC1.

Despite being in the same competition group as mAb114 (4), antibody 13C6 fails to neutralize EBOV due to its inability to remain bound to GP following cathepsin cleavage. Conversely, the Marburg GP-antibody MR78 recognizes an analogous receptor-binding domain on Marburg GP, and also binds to cleaved EBOV GP, yet fails to neutralize EBOV due to its inability to recognize the native trimer (uncleaved GP) (34, 35). The failure of MR78 to bind native EBOV GP led to the hypothesis that NPC1-blocking antibodies might not be elicited during Ebola virus infection (34). Strikingly, mAb114 overcomes these structural constraints by binding to the center of the GP1 chalice with a near vertical angle of approach (85° with respect to the viral membrane) that allows access to the GP1 core. The recently published crystal structure of NPC1-dC bound to GP_{THL} revealed that in addition to making contacts with the hydrophobic pocket exposed by glycan cap and MLD removal, NPC1 also contacts the surrounding charged region in $\beta 7$ – $\beta 9$ which is bound by mAb114 (Fig. S7B) (36). Taken together, these data reveal a key site of vulnerability in the EBOV GP targeted by mAb114 and demonstrate that this class of antibodies can be elicited by natural infection.

The experiments herein reveal that mAb100 and mAb114 mediate virus neutralization by targeting independent essential steps in EBOV entry: exposure of the RBD by protease cleavage and receptor binding. Since these steps are required for the entry of all members of the *Filoviridae* family (8, 11), our studies identify vulnerabilities targeted by the host immune system that could potentially be exploited in vaccine and therapeutic development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Binding requirements and structure of antibodies in complex with GP

(A) Schematic representation of GP monomer, colored by domain. GP1 core region (33–190) is colored blue, GP1 glycan cap is colored yellow (201–308), and the mucin-like domain is uncolored (309–501). The GP2 internal fusion loop (IFL) is colored red and the remainder of GP2 is colored orange. Glycans are shown as branched lines and proteolytic cleavage sites are labeled with arrows. Disulfide bonds within and between GP1 and GP2 are omitted for clarity. (B) Immunoprecipitation (IP) of soluble GP ectodomain containing or lacking the mucin-like domain (GP Δ Muc) by mAb100, mAb114 or isotype control. Binding and input were analyzed using immunoblotting for GP1. * Represents a GP1 degradation product present only in mucin-containing GP. (n=3, representative image shown) (C) Crystal structure of GP Δ Muc in ternary complex with Fab100 and Fab114. Fab100 is shown in purple (heavy chain) and white (light chain). Fab114 is shown in pink (heavy chain) and white (light chain). Molecular surfaces of two GP Δ Muc protomers are colored in green and beige, whereas the third is shown as a ribbon representation and colored according to the schematic in panel A.

Figure 2. Cryo-EM of GP_{Muc}-Fab100-Fab114 complex

Cryo-EM was performed on the ternary complex of GP_{Muc} with Fab100 and Fab114 at (A) pH 7.4 and (B) pH 5. Shown are the superimpositions of the crystal structure (ribbon) into the cryo-EM density maps at their respective pH.

Figure 3. Inhibition of cathepsin cleavage of GP by mAb100

(A) Fab100 binding occludes access to the β 13– β 14 loop of GP1. Protomers and Fab100 heavy and light chains are colored and oriented as in Fig. 1C. The variable domain of one Fab100 is shown in a ribbon representation and all other Fabs are removed for clarity. Inset shows a zoomed view of the Fab100–GP β 13– β 14 loop interface with the difference map generated by masking out the cryo-EM densities of the fitted crystal structures shown as a grey transparent surface. The β 13– β 14 loop is shown as a dashed yellow line connecting the GP1 core (blue) and the glycan cap (yellow). (B) GP_{Muc} was incubated with the indicated antibodies followed by cleavage at pH 5.5 by Cat L at 37 °C. Samples were removed at 5 minute intervals and analyzed by immunoblot for GP1. (n=3, representative image shown) (C) Binding kinetics of GP_{Muc} or GP_{THL} (THL) with Fab100 or KZ52 at the indicated pH as determined by biolayer interferometry. Equilibrium dissociation constants (K_D) are plotted on a negative log scale. (n=2, representative experiment shown)

Figure 4. Competition of NPC1 binding to GP by mAb114

(A) Fab114 binds to regions in the glycan cap and core of GP1. Protomers are colored as in Fig. 1C and viewed with a 100° rotation about the trimeric axis with respect to the orientation in Fig. 1C. The variable domain of a single Fab114 is shown in ribbons and all other Fabs have been removed for clarity. GP residues predicted to make contact with Fab114 are shown as transparent surfaces. (B) Immunoprecipitation of GP_{Muc} and GP_{THL} by the indicated antibodies. Samples were analyzed by immunoblot for GP1. (n=3, representative image shown) (C) Class averages of single particles from negative-stain electron micrographs of Fab114 in complex with GP_{Muc} and GP_{THL}. (D) Binding kinetics of GP_{Muc} or GP_{THL} with Fab114, 13C6 or monomeric domain C of NPC1 (NPC1-dC) at the indicated pH as measured by biolayer interferometry. Equilibrium dissociation constants (K_D) are plotted on a negative log scale. * indicates no binding. (n=2, representative experiment shown) (E) Inhibition of NPC1-dC binding to GP_{THL} by competitor proteins (NPC1-dC) or antibodies (mAb100, mAb114, 13C6, KZ52, isotype control) was determined by biolayer interferometry. Dashed line represents 60% inhibition of binding. (n=3, representative experiment shown)