Crystal Structure of Marburg Virus GP Bound to a Cross-Reactive Antibody from a Human Survivor

Filoviruses, including Marburg and Ebola viruses, cause hemorrhagic fever with up to 90% mortality in humans. Despite its potential availability, no cross-reactive antibody has been reported against filovirus glycoproteins (GPs). Here, we present the structure of a cross-reactive antibody MR78, derived from a human survivor, in complex with Marburg virus (MARV) GP. The conformational epitope recognized by MR78 is shown to overlap with the receptor binding site on GP, and is highly conserved among all filoviruses. The GP and Fab complex structure provides a clue for structure-based vaccine designs.

Marburg virus (MARV) and Ebola virus (EBOV), members of the family *Filoviridae*, cause highly lethal hemorrhagic fever in humans. EBOV reemerged and caused the biggest outbreak of a filovirus in Western Africa, mainly in Guinea, Liberia, and Sierra Leone, in 2014 [1]. MARV also caused the biggest outbreak in Angola in 2004–2005, which exhibited ~90% mortality [2, 3]. Currently, no licensed therapeutic agents or vaccines are available. Filoviruses possess a sole GP on their envelope (lipid bilayer membrane), which is mainly comprised of the highly glycosylated GP1, responsible for receptor attachment, and the GP2, mediating membrane fusion. To enter a cell, a filovirus is initially internalized by macropinocytosis. Next, cathepsin cleavage of GPs exposes the receptor-binding site (RBS) fully for the Niemann Pick C1 (NPC1) receptor in the endosome.

Therapeutic antibodies are expected to be a highly effective treatment not only for infectious diseases but also for various other diseases. Recently, broadly cross-reactive antibodies have been elicited against the glycoproteins of HIV and Influenza virus (Flu) as potentially therapeutic agents, and the structural basis for the broad cross reactivity has also been solved [4-6]. So far, however, little has been known about cross-reactive antibodies against MARV and EBOV. Especially, structural information for such monoclonal antibodies (mAbs) is lacking in filovirus research, in contrast to those of HIV and Flu. Here, we report the structures of the cross-reactive antibody, MR78, in complex with MARV GP. Our structural study sheds light on the humoral response and vaccine development by revealing how MR78 provides cross reactivity against filovirus GPs [7, 8].

For a structural understanding of the cross reactivity by MR78, we determined the crystal structure of the antigen-binding fragment (Fab) MR78 in complex with MARV GP (Fig. 1). The GP was in its supposed metastable, prefusion conformation. Diffraction to 3.6 Å resolution was obtained from the cocrystal of MARV GP and Fab MR78, which contained four complexes in the asymmetric unit. The overall structure of MR78 and MARV GP exhibited inverted tripod-like binding modes [Fig. 1(a)]. Although MARV and EBOV differ in the whole GP sequences by ~70%, MR78 recognizes the same regions on both viruses. The epitope recognized by MR78 is located only on the GP1 subunit. This is different from previously reported ebolavirus GP-binding antibodies, KZ52, 16F6, 2G4 and 4G7, which bind both GP1 and GP2 subunits [Fig. 1(b)] [9-12]. Unlike ebolavirus GP-binding antibodies, MR78 could absolutely inhibit domain binding of the NPC1 domain C. The binding site of MR78 would be on or close to the putative RBS.

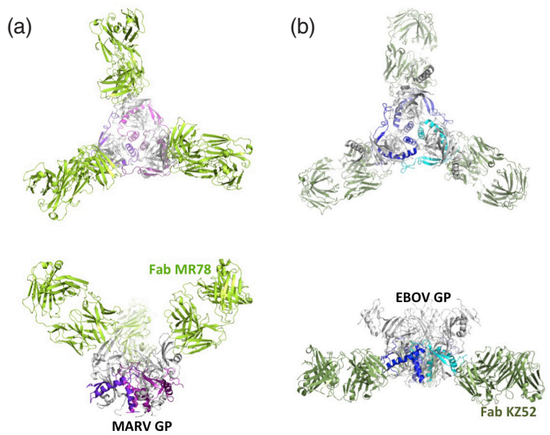

Figure 1: Crystal structures of filovirus GPs and antibodies. Top view (top) and side view (bottom). (a) MARV GP and Fab MR78 (light green). (b) EBOV GP and Fab KZ52 (dark green).

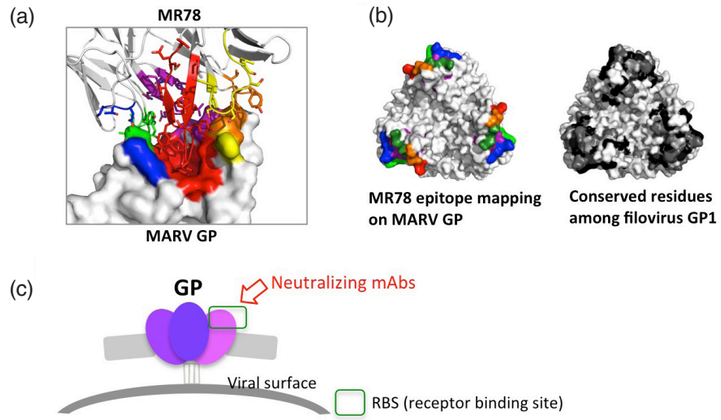

Figure 2: Binding site of MR78 and MARV GP. (a) The interaction between Fab MR78 (ribbon model) and MARV GP (surface). CDR-L1 (green), CDR-L2 (blue), CDR-L3 (purple), CDR-H1 (yellow), CDR-H2 (orange), CDR-H3 (red). (b) The epitope recognized by MR78 and the conserved residues (indicating by black, dark gray and gray depending on the level of conservation) on filovirus GP trimer. (c) Schematic presentation of MARV GP and its neutralizing antibodies.

To assess the MR78-epitope conservation among filovirus GPs, the protein sequence alignment was performed in ebolaviruses (*Zaire, Sudan, Reston, Taï Forest, Bundibugyo*), marburgviruses (Musoke, Angola, Popp, Ci67, DRC1999, Ravn), and cuevaviruses (Lloviu). As a result, 85% of the residues comprising the MR78 epitope showed similarity among filoviruses. Especially, the highly conserved residues on GP were found to interact with both heavy and light chains, however, the major interaction is comprised of the residues in the third complementarity-determining region of the heavy chain (CDR-H3) of Fab MR78 [Fig. 2(a)]. By contrast, the difference is ~70% in the whole GPs between MARV and EBOV as described above. These results explain why MR78 shows cross reactivity between MARV and EBOV. It is thus suggested that MR78 might bind to all filovirus GPs and inhibit the NPC1 binding [Fig. 2(b)].

The highly conserved MR78 epitope provided the structural basis for the cross reactivity against MARV and EBOV. However, no pan-reactive anti-filovirus antibodies have yet been reported from immunization using EBOV GP. MARV GP might be a better antigen to elicit cross-reactive mAbs against all filoviruses. The anti-RBS antibodies, like MR78, would be promising therapeutic candidates, because the escape mutants from anti-RBS antibodies would lose the receptor binding ability and then not be able to survive [Fig. 2(c)].

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